



Boehm,
Richard A. Heyman

PATENT
203/270

S P E C I F I C A T I O N

COMPOUNDS HAVING SELECTIVE ACTIVITY FOR RETINOID X RECEPTORS, AND MEANS FOR MODULATION OF PROCESSES MEDIATED BY RETINOID X RECEPTORS

RELATED APPLICATIONS

(Ansdi) This application is a continuation-in-part of the application Serial No. 08/052,051 filed on April 21, 1993, which is a continuation-in-part of the application Serial No. 08/027,747 filed on March 5, 1993, which is a continuation-in-part of application Serial No. 08/003,223 filed on January 11, 1993, which is a continuation-in-part of application Serial No. 01/944,783 filed on September 11, 1992, which is a continuation-in-part of application Serial No. 0872,707 filed April 22, 1992, whose entire disclosures are incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to intracellular receptors and ligands therefor. More specifically, this invention relates to compounds having selective activity for specific retinoic acid receptors, and methods for use of such compounds.

BACKGROUND OF THE INVENTION

The vitamin A metabolite retinoic acid has long been recognized to induce a broad spectrum of biological effects. A variety of structural analogues of retinoic acid have been synthesized that also have been found to be bioactive. Some, such as Retin-A® (registered trademark of Johnson & Johnson) and

2

Accutane® (registered trademark of Hoffmann-LaRoche), have found utility as therapeutic agents for the treatment of various pathological conditions. Metabolites of vitamin A and their synthetic analogues are collectively herein called "retinoids".

5 Synthetic retinoids have been found to mimic many of the pharmacological actions of retinoic acid. However, the broad spectrum of pharmacological actions of retinoic acid is not reproduced in full by all bioactive synthetic retinoids.

10 Medical professionals have become very interested in the medicinal applications of retinoids. Among their uses approved by the FDA is the treatment of severe forms of acne and psoriasis. A large body of evidence also exists that these compounds can be used to arrest and, to an extent, reverse the 15 effects of skin damage arising from prolonged exposure to the sun. Other evidence exists that these compounds may be useful in the treatments of a variety of severe cancers including melanoma, cervical cancer, some forms of leukemia, and basal and squamous cell carcinomas. Retinoids have also shown an ability to be efficacious in treating premalignant cell lesions, such as oral 20 leukoplakia, and to prevent the occurrence of malignancy.

Use of the retinoids is associated with a number of significant side effects. The most serious of these is that, as a class, they are among the most potent teratogens known.

25 Teratogens are compounds that cause severe birth defects during specific periods of fetal exposure. Other side effects include irritation of the tissues treated, which can be so severe that patients cannot tolerate treatment.

30 Various investigations have been undertaken to elucidate the structure-activity relationships governing the abilities of synthetic retinoids to induce the various pharmacological consequences of retinoic acid exposure. This has

been a complicated task, however, since the assays available to investigators have been bioassays, carried out either in intact animals or in isolated tissues. Technical constraints have often dictated the use of different small animal species for different assays. Interpretation of results has been complicated by possible pharmacokinetic and metabolic effects and possible species differences in the receptors involved. Nevertheless, definite differences in the pharmacological effects of various synthetic retinoids have been observed.

Major insight into the molecular mechanism of retinoic acid signal transduction was gained in 1988. Prior to that time, several high abundance cellular retinoid binding proteins were incorrectly inferred to be the signal transducing receptors for retinoic acid. In 1988, a member of the steroid/thyroid hormone intracellular receptor superfamily (Evans, *Science*, 240:889-95 (1988)) was shown to transduce a retinoic acid signal (Giguere et al., *Nature*, 330:624-29 (1987); Petkovich et al., *Nature*, 330: 444-50 (1987)). This unexpected finding related retinoic acid to other non-peptide hormones and elucidated the mechanism of retinoic acid effects in altering cell function. It is now known that retinoids regulate the activity of two distinct intracellular receptor subfamilies; the Retinoic Acid Receptors (RARs) and the Retinoid X Receptors (RXRs).

The first retinoic acid receptor identified, designated RAR-alpha, acts to modulate transcription of specific target genes in a manner which is ligand-dependent, as has been shown to be the case for many of the members of the steroid/thyroid hormone intracellular receptor superfamily. The endogenous low-molecular-weight ligand upon which the transcription-modulating activity of RAR-alpha depends is all-*trans*-retinoic acid.

Retinoic acid receptor-mediated changes in gene expression result in characteristic alterations in cellular phenotype, with consequences in many tissues manifesting the biological response to retinoic acid. Two additional genes closely related to RAR-alpha were recently identified and were designated RAR-beta and RAR-gamma and are very highly related (Brand *et al.*, *Nature*, 332:850-53 (1988); Ishikawa *et al.*, *Mol. Endocrin.*, 4:837-44 (1990)). In the region of the retinoid receptors which can be shown to confer ligand binding, the primary amino acid sequences diverge by less than 15% among the three RAR subtypes or isoforms. All-*trans*-retinoic acid is a natural ligand for the retinoic acid receptors (RARs) and is capable of binding to these receptors with high affinity, resulting in the regulation of gene expression. The newly-discovered retinoid metabolite, 9-*cis*-retinoic acid, is also an activator of RARs.

A related but unexpected observation was made recently (Mangelsdorf *et al.*, *Nature*, 345:224-29 (1990)), in which another member of the steroid/thyroid receptor superfamily was also shown to be responsive to retinoic acid. This new retinoid receptor subtype has been designated Retinoid X Receptor (RXR), because certain earlier data suggested that a derivative of all-*trans*-retinoic acid may be the endogenous ligand for RXR. Like the RARs, the RXRs are also known to have at least three subtypes or isoforms, namely RXR-alpha, RXR-beta, and RXR-gamma, with corresponding unique patterns of expression (Mangelsdorf *et al.*, *Genes & Devel.*, 6:329-44 (1992)).

Although both the RARs and RXRs respond to all-*trans*-retinoic acid *in vivo*, the receptors differ in several important aspects. First, the RARs and RXRs are significantly divergent in primary structure (e.g., the ligand binding domains of RAR α and

RXR α have only 27% amino acid identity). These structural differences are reflected in the different relative degrees of responsiveness of RARs and RXRs to various vitamin A metabolites and synthetic retinoids. In addition, distinctly different 5 patterns of tissue distribution are seen for RARs and RXRs. For example, in contrast to the RARs, which are not expressed at high levels in the visceral tissues, RXR α mRNA has been shown to be most abundant in the liver, kidney, lung, muscle and intestine. Finally, the RARs and RXRs have different target gene 10 specificity. For example, response elements have recently been identified in the cellular retinal binding protein type II (CRBPII) and apolipoprotein AI genes which confer responsiveness to RXR, but not RAR. Furthermore, RAR has also been recently shown to repress RXR-mediated activation through the CRBPII RXR 15 response element (Mangelsdorf *et al.*, *Cell*, 66:555-61 (1991)). These data indicate that two retinoic acid responsive pathways are not simply redundant, but instead manifest a complex 20 interplay. Recently, Heyman *et al.* (*Cell*, 68:397-406 (1992)) and Levin *et al.* (*Nature*, 355:359-61 (1992)) independently demonstrated that 9-cis-retinoic acid is a natural endogenous ligand for the RXRs. 9-cis-retinoic acid was shown to bind and transactivate the RXRs, as well as the RARs, and therefore appears to act as a "bifunctional" ligand.

In view of the related, but clearly distinct, nature of 25 these receptors, ligands which are more selective for the Retinoid X Receptor subfamily would be of great value for selectively controlling processes mediated by one or more of the RXR isoforms, and would provide the capacity for independent control of the physiologic processes mediated by the RXRs. 30 Ligands which preferentially affect one or more but not all of

the receptor isoforms also offer the possibility of increased therapeutic efficacy when used for medicinal applications.

The entire disclosures of the publications and references referred to above and hereafter in this specification
5 are incorporated herein by reference.

SUMMARY OF THE INVENTION

The present invention is directed to compounds, compositions, and methods for modulating processes mediated by one or more Retinoid X Receptors. More particularly, the
10 invention relates to compounds which selectively or preferentially activate Retinoid X Receptors, in comparison to Retinoic Acid Receptors. These compounds selectively modulate processes mediated by Retinoid X Receptors. Accordingly, the invention also relates to methods for modulating processes
15 selectively mediated by one or more Retinoid X Receptors, in comparison to Retinoic Acid Receptors, by use of the compounds of this invention. Examples of compounds used in and forming part of the invention include bicyclic benzyl, pyridinyl, thiophene, furanyl, and pyrrole derivatives. Pharmaceutical compositions
20 containing the compounds disclosed are also within the scope of this invention. Also included are methods for identifying or purifying Retinoid X Receptors by use of the compounds of this invention.

BRIEF DESCRIPTION OF THE FIGURES

The present invention may be better understood and its
25 advantages appreciated by those skilled in the art by referring to the accompanying drawings wherein:

Figure 1 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNCB.

5 Figure 2 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by all-*trans*-retinoic acid.

Figure 3 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 9-*cis*-retinoic acid.

10 Figure 4 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNEB.

15 Figure 5 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-bromo-TTNEB.

Figure 6 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNCHBP.

20 Figure 7 presents the standardized dose response profiles showing the transactivation of RAR and RXR isoforms by 3-methyl-TTNEHBP.

Figure 8 presents the inhibition of transglutaminase activity by 9-*cis*-retinoic acid, all-*trans*-retinoic acid, and 3-methyl-TTNCB.

25 Figure 9 presents the Topical Dose Response, based on the test on Rhino mice, for 9-*cis*-retinoic acid, all-*trans*-retinoic acid, 3-methyl-TTNCB, 1, 25-dihydroxy Vitamin D.

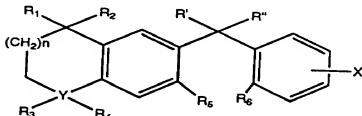
30 Figure 10 presents the effect on rat HDL cholesterol of all-*trans*-retinoic acid, 9-*cis*-retinoic acid, 3-methyl-TTNCB, and 3-methyl-TTNEB.

Figure 11 presents the concentration-related effect of 3-methyl-TTNEB and TTNPB individually on incorporation of radiolabeled thymidine into DNA.

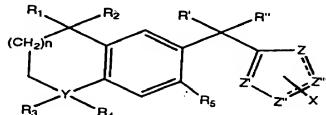
5 Figure 12 presents the concentration-related effect of a combination of 3-methyl-TTNEB and TTNPB on incorporation of radiolabeled thymidine into DNA.

DETAILED DESCRIPTION OF THE INVENTION

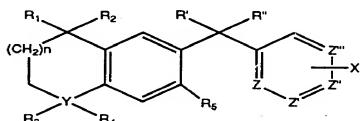
This invention discloses retinoid-like compounds or ligands which have selective activity for members of the subfamily of Retinoid X Receptors (RXRs), in comparison to members of the subfamily of Retinoic Acid Receptors (RARs). Examples of such compounds are bicyclic benzyl, pyridinyl, thiophene, furanyl, and pyrrole derivatives which can be represented by the formulae:



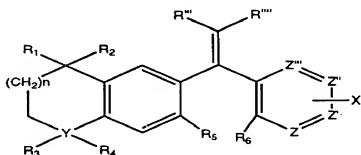
or



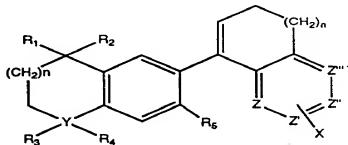
or



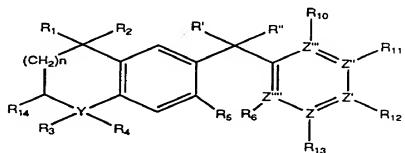
or



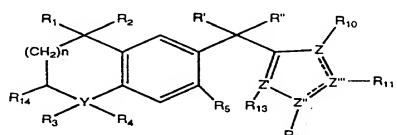
or



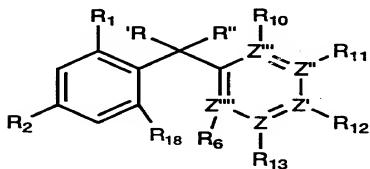
OR



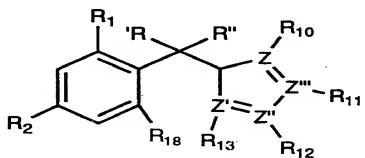
OR



OR



OR



wherein

R₁ and R₂, each independently, represent hydrogen or lower alkyl or acyl having 1-4 carbon atoms;

5 Y represents C, O, S, N, CHO_H, CO, SO, SO₂, or a pharmaceutically acceptable salt;

R₃ represents hydrogen or lower alkyl having 1-4 carbon atoms where Y is C or N;

10 R₄ represents hydrogen or lower alkyl having 1-4 carbon atoms where Y is C, but R₄ does not exist if Y is N, and neither R₃ or R₄ exist if Y is S, O, CHO_H, CO, SO, or SO₂;

R' and R" represent hydrogen, lower alkyl or acyl having 1-4 carbon atoms, OH, alkoxy having 1-4 carbon atoms, thiol or thioether, or amino,

15 or R' or R" taken together form an oxo (keto), methano, thioketo, HO-N=, NC-N=, (R₇R₈)N-N=, R₁₇O-N=, R₁₇N=, epoxy, cyclopropyl, or cycloalkyl group and wherein the epoxy, cyclopropyl, and cycloalkyl groups can be substituted with lower alkyl having 1-4 carbons or halogen;

20 R' and R" represent hydrogen, halogen, lower alkyl or acyl having 1-4 carbon atoms, alkyl amino,

or R' and R" taken together form a cycloalkyl group having 3-10 carbons, and wherein the cycloalkyl group can be substituted with lower alkyl having 1-4 carbons or halogen;

25 R₅ represents hydrogen, a lower alkyl having 1-4 carbons, halogen, nitro, OR₇, SR₇, NR₇R₈, or (CF)_nCF₃, but R₅ cannot be hydrogen if together R₆, R₁₀, R₁₁, R₁₂ and R₁₃ are all hydrogen, Z, Z', Z'', and Z''' are all carbon, and R' and R" represent H, OH, C₁-C₄ alkoxy or C₁-C₄ acyloxy or R' and R" taken together form an oxo, methano, or hydroxyimino group;

30 R₆, R₁₀, R₁₁, R₁₂, R₁₃ each independently represent hydrogen, a lower alkyl having 1-4 carbons, halogen, nitro, OR₇, SR₇, NR₇R₈ or

(CF)_nCF₃, and exist only if the Z, Z', Z'', Z''', or Z'''' from which it originates is C, or each independently represent hydrogen or a lower alkyl having 1-4 carbons if the Z, Z', Z'', Z''', or Z'''' from which it originates is N, and where one of R₆, R₁₀, R₁₁, R₁₂ or R₁₃ is

5 X;

R₇ represents hydrogen or a lower alkyl having 1-6 carbons;

R₈ represents hydrogen or a lower alkyl having 1-6 carbons;

R₉ represents a lower alkyl having 1-4 carbons, phenyl, aromatic alkyl, or q-hydroxyphenyl, q-bromophenyl, q-chlorophenyl, 10 q-florophenyl, or q-iodophenyl, where q=2-4;

R₁₄ represents hydrogen, a lower alkyl having 1-4 carbons, oxo, hydroxy, acyl having 1-4 carbons, halogen, thiol, or thioketone;

R₁₇ represents hydrogen, lower alkyl having 1-8 carbons, alkenyl (including halogen, acyl, OR₇ and SR₇ substituted alkenes), 15 R₉, alkyl carboxylic acid (including halogen, acyl, OR₇ and SR₇ substituted alkyls), alkenyl carboxylic acid (including halogen, acyl, OR₇ and SR₇ substituted alkenes), alkyl amines (including halogen, acyl, OR₇ and SR₇ substituted alkyls), and alkenyl amines (including halogen, acryl, OR₇ and SR₇ substituted alkenes);

20 R₁₈ represents hydrogen, a lower alkyl having 1-4 carbons, halogen, nitro, OR₇, SR₇, NR₇R₈, or (CF)_nCF₃;

X is COOH, tetrazole, PO₃H, SO₃H, CHO, CH₂OH, CONH₂, COSH, COOR₉, COSR₉, CONHR₉, or COOW where W is a pharmaceutically acceptable salt, and where X can originate from any C or N on the 25 ring;

Z, Z', Z'', Z''' and Z'''', each independently, represent C, S, O, N, or a pharmaceutically acceptable salt, but is not O or S if attached by a double bond to another such Z or if attached to another such Z which is O or S, and is not N if attached by a single bond to another such Z which is N;

30 n = 0-3; and

the dashed lines in the second and seventh structures shown depict optional double bonds.

As used in this disclosure, pharmaceutically acceptable salts include but are not limited to: hydrochloric, hydrobromic, 5 hydroiodic, hydrofluoric, sulfuric, citric, maleic, acetic, lactic, nicotinic, succinic, oxalic, phosphoric, malonic, salicylic, phenylacetic, stearic, pyridine, ammonium, piperazine, diethylamine, nicotinamide, formic, urea, sodium, potassium, calcium, magnesium, zinc, lithium, cinnamic, methylamino, 10 methanesulfonic, picric, tartaric, triethylamino, dimethylamino, and tris(hydroxymethyl)aminomethane. Additional pharmaceutically acceptable salts are known to those of skill in the art.

Representative derivatives according to the present invention include the following:

15 p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-carbonyl)]-benzoic acid, also known as 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid, and designated "3-methyl-TTNCB";
20 p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-isopropyl-2-naphthyl-(2-carbonyl)]-benzoic acid, also known as 4-[(3-isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid, and designated "3-IPR-TTNCB" or Compound 37;
25 p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-isopropyl-2-naphthyl-(2-methano)]-benzoic acid, also known as 4-[1-(3-isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, and designated "3-IPR-TTNEB" or Compound 42;
p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-ethyl-2-naphthyl-(2-methano)]-benzoic acid, also known as 4-[1-(3-ethyl-5,5,8,8-

5 tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, and
designated "3-ethyl-TTNEB" or Compound 45;

5 p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-bromo-2-naphthyl-
(2-methano)]-benzoic acid, also known as 4-[1-(3-bromo-5,5,8,8-
5 tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, and
designated "3-bromo-TTNEB" or Compound 46;

10 p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-chloro-2-naphthyl-
(2-methano)]-benzoic acid, also known as 4-[1-(3-chloro-5,5,8,8-
tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, and
10 designated "3-chloro-TTNEB" or Compound 43;

15 p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-
methano)]-benzoic acid, also known as 4-[1-(3,5,5,8,8-pentamethyl-
5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid, and designated
"3-methyl-TTNEB";

20 p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-
hydroxymethyl)]-benzoic acid, also known as 4-[1-(3,5,5,8,8-
pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)hydroxymethyl]benzoic
acid, and designated "3-methyl-TTNHMB";

25 p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-bromo-2-naphthyl-
(2-carbonyl)]-benzoic acid, also known as 4-[(3-bromo-5,5,8,8-
tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid,
and designated "3-bromo-TTNCB" or Compound 41;

30 p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-chloro-2-naphthyl-
(2-carbonyl)]-benzoic acid, also known as 4-[(3-chloro-5,5,8,8-
tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid,
and designated "3-chloro-TTNCB" or Compound 38;

35 p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-hydroxy-2-naphthyl-
(2-carbonyl)]-benzoic acid, also known as 4-[(3-hydroxy-5,5,8,8-
tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzoic acid,
30 and designated "3-hydroxy-TTNCB" or Compound 39;

p[5,5,8,8-tetramethyl-1,2,3,4-tetrahydro-3-ethyl-2-naphthyl-(2-carbonyl)]-benzoic acid, also known as 4-[(3-ethyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl) carbonyl]benzoic acid, and designated "3-ethyl-TTNCB" or Compound 40;

5 p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-thioketo)]-benzoic acid, also known as 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl) thioketo]benzoic acid, and designated "thioketone";

10 p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-carbonyl)]-N-(4-hydroxyphenyl)benzamide, also known as 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl) carbonyl]-N-(4-hydroxyphenyl)benzamide, and designated "3-methyl-TTNCHBP";

15 p[3,5,5,8,8-pentamethyl-1,2,3,4-tetrahydro-2-naphthyl-(2-methano)]-N-(4-hydroxyphenyl)benzamide, also known as 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]-N-(4-hydroxyphenyl)benzamide, and designated "3-methyl-TTNEHBP" or Compound 63;

20 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]pyridine-5-carboxylic acid, designated "TPNEP" or Compound 58;

ethyl 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]pyridine-5-carboxylate, designated "TPNEPE" or Compound Et-58;

25 2-[1-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]pyridine-5-carboxylic acid, designated "TTNEP" or Compound 56;

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)epoxy]benzoic acid, designated "TPNEB" or Compound 47;

30 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)cyclopropyl]benzoic acid, designated "TPNCB" or Compound 48;

4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzenetetrazole, designated "3-methyl-TTNEBT" or Compound 55;

5 5-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]pyridine-2-carboxylic acid, designated "TPNEPC" or Compound 60;

10 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)cyclopropyl]pyridine-5-carboxylic acid, designated "TPNCP" or Compound 62;

15 methyl 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)cyclopropyl]pyridine-5-carboxylate, designated Compound Me-62;

20 4-[1-(2-methyl-4-t-butylphenyl)ethenyl] benzoic acid, designated Compound 133;

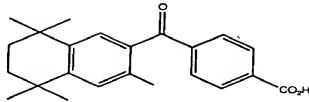
15 4-[1-(2-methyl-4-t-butylphenyl)cyclopropyl] benzoic acid, designated Compound 135;

25 4-[(2-methyl-4-t-butylphenyl)carbonyl] benzoic acid, designated Compound 136;

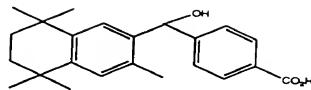
4-[(2-methyl-4-t-butylphenyl)carbonyl] benzoic acid oxime, 20 designated Compound 137; and

4-[1-(2-methyl-4-t-butylphenyl)carbonyl] benzoic acid methyloxime, designated Compound 144.

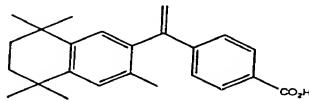
Representative structures for such compounds are as follows:



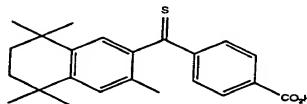
3-methyl-TTNNCB



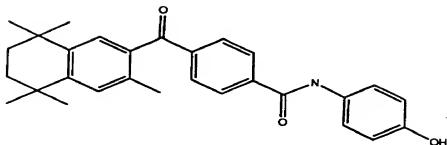
3-methyl-TTNHMB



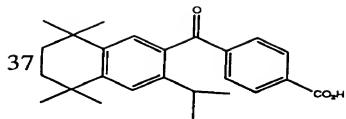
3-methyl-TTNEB



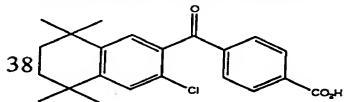
thioketone



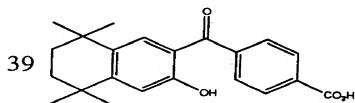
3-methyl-TTNCHBP



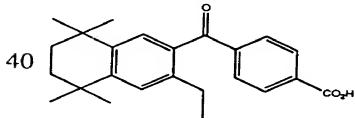
4-[(3-isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl] benzoic acid (3-Pr-TTNCB)



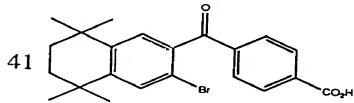
4-[(3-chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl] benzoic acid (3-chloro-TTNCB)



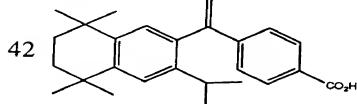
4-[(3-hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl] benzoic acid (3-hydroxy-TTNCB)



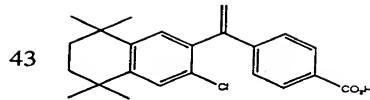
4-[(3-ethyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl] benzoic acid (3-Et-TTNCB)



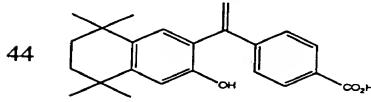
4-[(3-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl] benzoic acid (3-bromo-TTNCB)



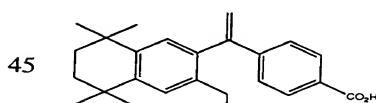
4-[(3-isopropyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl] benzoic acid (3-Pr-TTNEB)



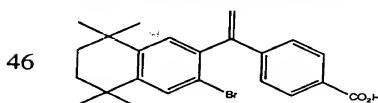
4-[(1-(3-chloro-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl)benzoic acid (3-chloro-TTNEB)



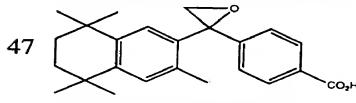
4-[(1-(3-hydroxy-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl)benzoic acid (3-hydroxy-TTNEB)



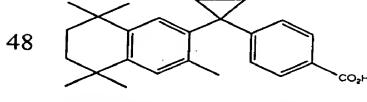
4-[(1-(3-ethyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl)benzoic acid (3-Et-TTNEB)



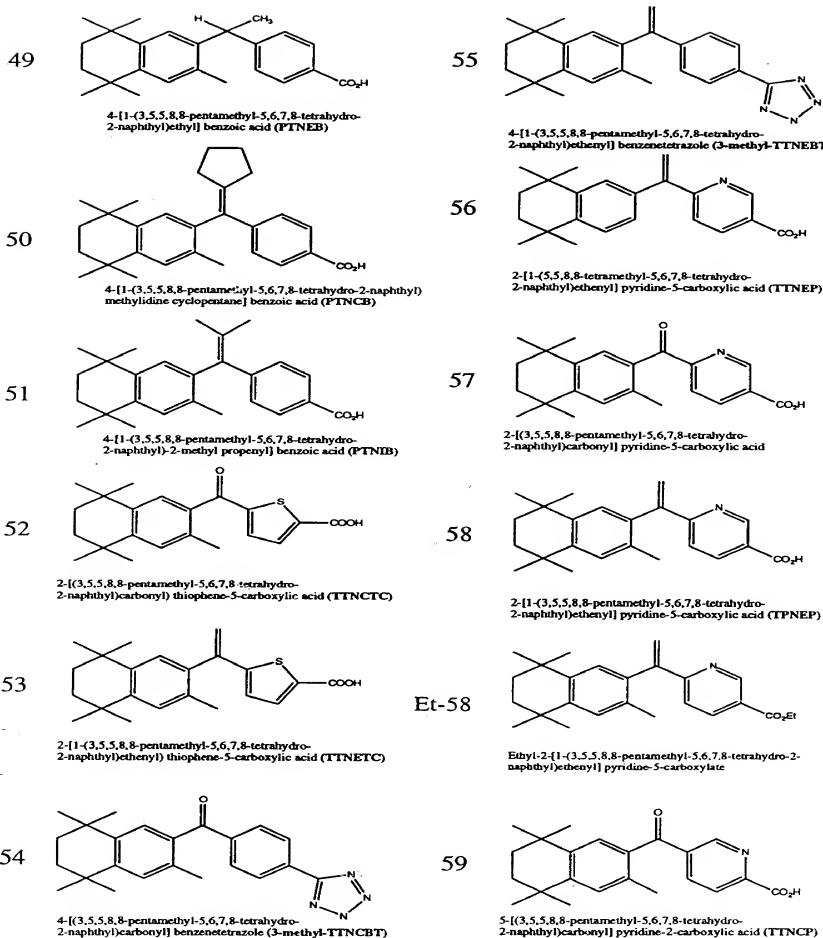
4-[(1-(3-bromo-5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl)benzoic acid (3-bromo-TTNEB)

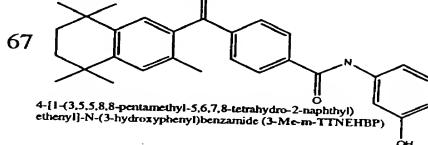
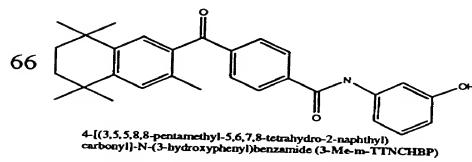
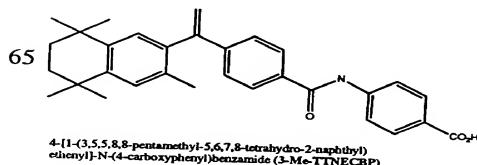
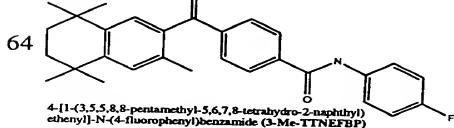
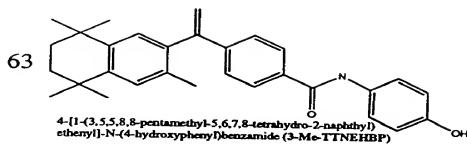
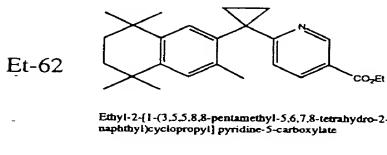
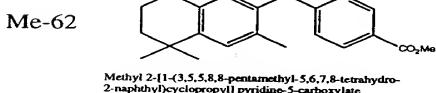
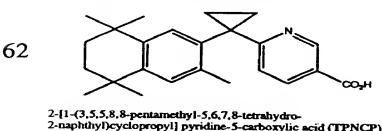
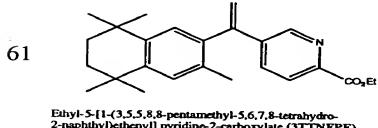
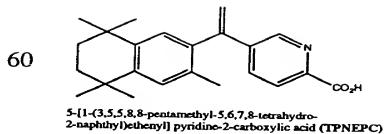


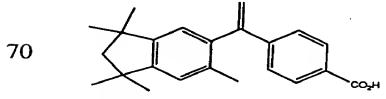
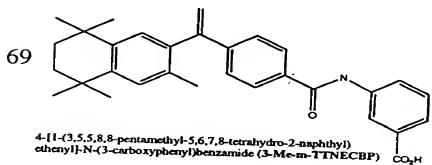
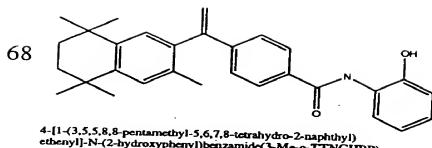
4-[(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)epoxy)benzoic acid (TPNEB)



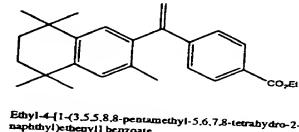
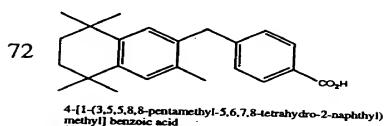
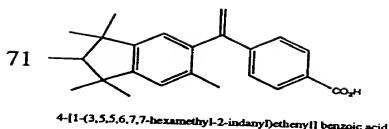
4-[(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)cyclopropyl)benzoic acid (TPNCB)



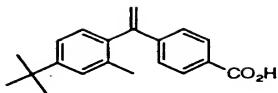




Et-3-methyl-TTNEB

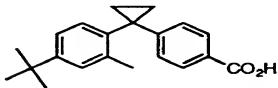


133



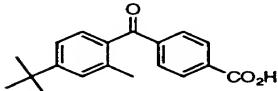
4-[1-(2-methyl-4-t-butylphenyl)ethenyl]
benzoic acid

135



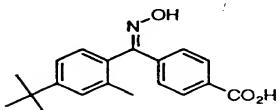
4-[1-(2-methyl-4-t-butylphenyl)cyclopropyl]
benzoic acid

136



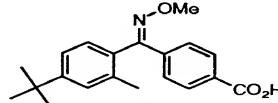
4-[(2-methyl-4-t-butylphenyl)carbonyl]
benzoic acid

137



4-[1-(2-methyl-4-t-butylphenyl)carbonyl]
benzoic acid oxime

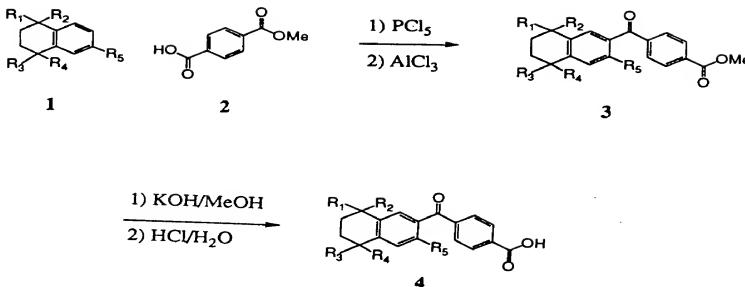
144



4-[1-(2-methyl-4-t-butylphenyl)carbonyl]
benzoic acid methyloxime

In addition, thiophene, furanyl, pyridine, pyrazine, pyrazole, pyridazine, thadiazole, and pyrrole groups function as isosteres for phenyl groups, and may be substituted for the phenyl group of the above bicyclic benzyl derivatives.

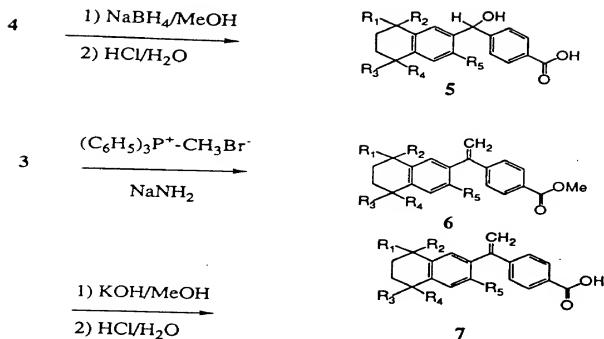
5 Representative derivatives of the present invention can be prepared according to the following illustrative synthetic schemes:



Compounds of structure 1 containing R_5 = lower alkyl are prepared in accordance with United States Patent No. 2,897,237. When R_5 = Halo, OH, amino, or thio, the products are prepared by standard Friedel-Crafts reaction conditions combining the appropriate substituted benzene with 2,5-dichloro-2,5-dimethyl hexane in the presence of aluminum trichloride.

Condensation of 1 with mono-methyl terephthalate 2 was carried out by addition of PCl_5 to 1 and 2 in CH_2Cl_2 , followed by addition of AlCl_3 , at room temperature.

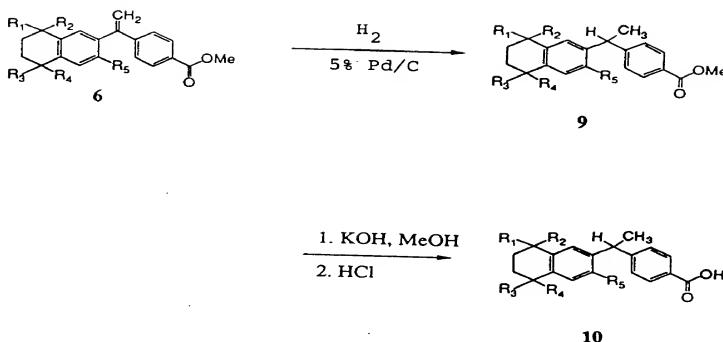
The resulting methyl esters 3 are hydrolyzed to the carboxylic acid 4 by refluxing in aqueous $\text{KOH}-\text{MeOH}$ followed by acidification.



Treatment of ketone **4** with NaBH_4 afforded alcohol **5**

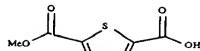
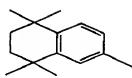
Treatment of the methyl ester **3** with methyltriphosphonium bromide-sodium amide in THF afforded the methano compound **6**.

The carboxylic acid 7 was formed by adding KOH to methanolic compound 6 in MeOH, followed by acidification.

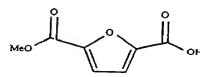
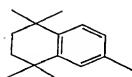
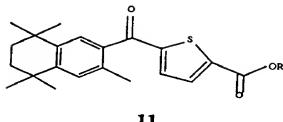


Treatment of the methyl ester **6** with hydrogen gas and 5% palladium over carbon in ethyl acetate yields the hydrogenated compound **9**.

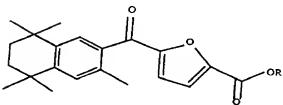
Treatment of compound **9** with aqueous KOH in refluxing MeOH, followed by acidification, yields the carboxylic acid compound **10**.



1) PCl_5
2) AlCl_3

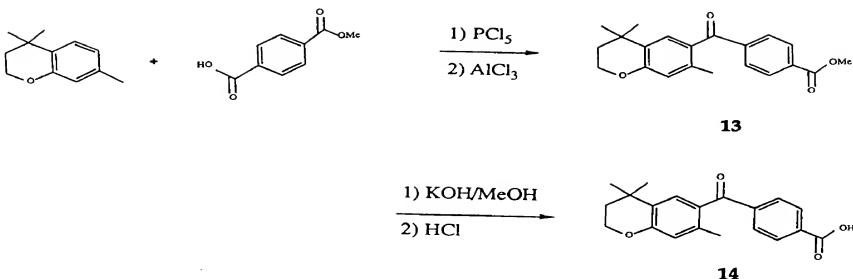


1) PCl_5
2) AlCl_3

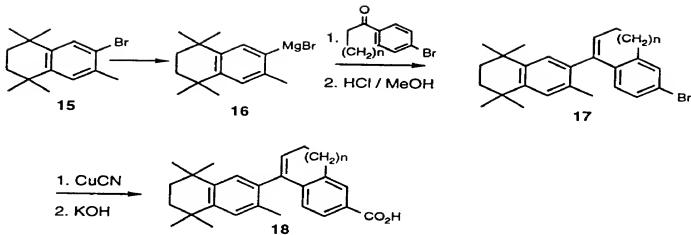


R = Me or H

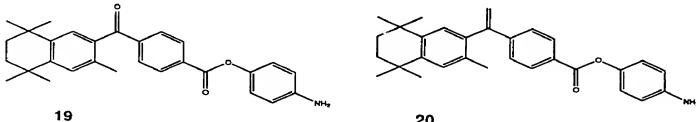
Condensation of 1 with thiophene 2,5-mono methyl dicarboxylic acid or furanyl 2,5-mono methyl dicarboxylic acid was carried out by addition of PCl_5 in CH_2Cl_2 , followed by addition of 5 AlCl_3 at room temperature to give esters 11 and 12, which were hydrolyzed with KOH followed by acidification to the corresponding acids.



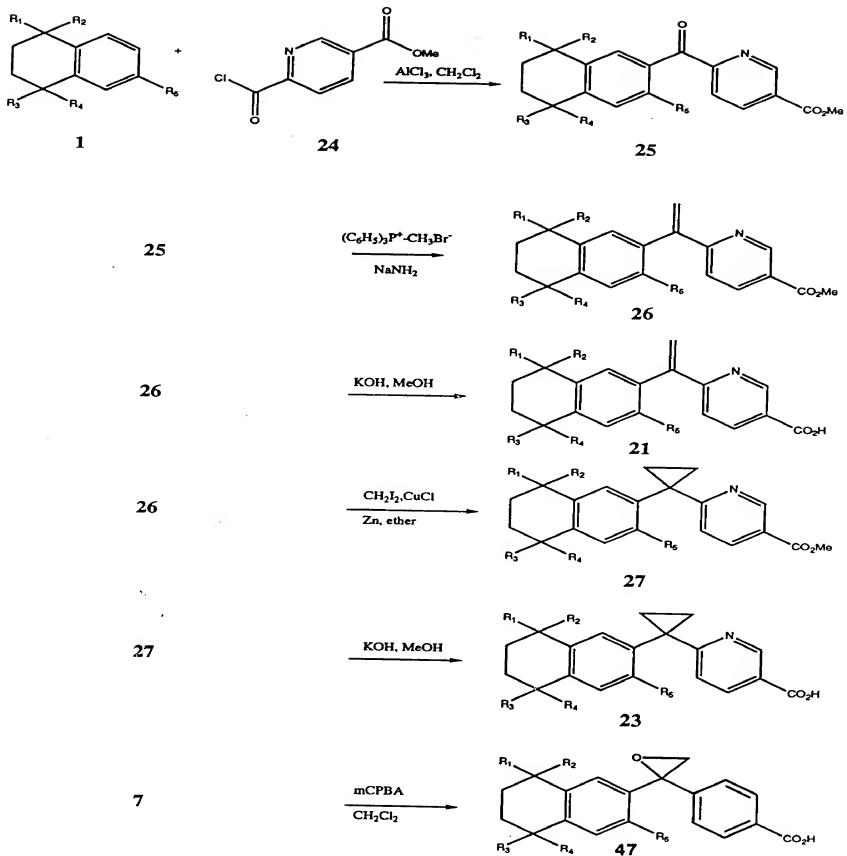
4,4-Dimethylchroman and 4,4-dimethyl-7-alkylchroman compounds of type 13 and 14 as well as 4,4-dimethylthiochroman, 4,4-dimethyl-7-alkylthiochroman, 4,4-dimethyl-1,2,3,4-tetrahydroquinoline, and 4,4-dimethyl-7-alkyl-1,2,3,4-tetrahydroquinoline analogs were synthesized by similar methods as compound 3, i.e., Friedel-Crafts conditions combining the appropriate dimethylchroman, dimethylthiochroman or dimethyltetrahydroquinoline with mono-methyl terephthalate acid chloride in the presence of AlCl_3 or SnCl_4 , followed by base hydrolysis and acidification to give the carboxylic acid. For the synthesis of the tetrahydroquinoline analogs, it was necessary to acylate the amine before Friedel-Crafts coupling with mono-methyl terephthalate acid chloride. For the synthesis of the appropriate dimethylchromans, dimethylthiochromans and tetrahydroquinolines, see U.S. Patent Nos. 5,053,523 and 5,023,341 and European Patent Publication No. 0284288.



Compounds of the type 18 were synthesized by nucleophilic addition of the Grignard reagent 16 to bromotetralone, bromoindane, or other 5 bicyclic ketone derivative. Treatment of the resulting alcohol with methanolic HCl gave the intermediate 17. Displacement of the bromine with CuCN in quinoline gave the nitrile which was then hydrolyzed to the acid 18 in refluxing KOH. Bromine compound 15 was synthesized from 2,5-dichloro-2,5-dimethylhexane and 2-10 bromotoluene with a catalytic amount of AlCl_3 .



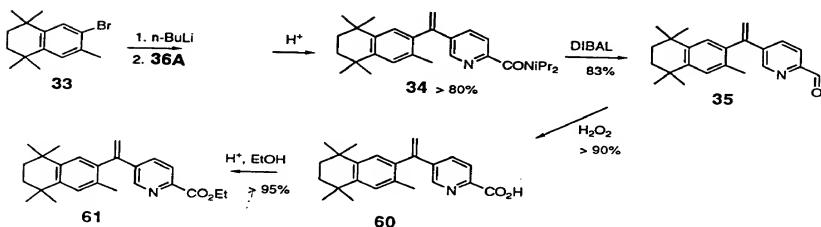
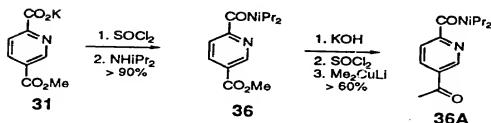
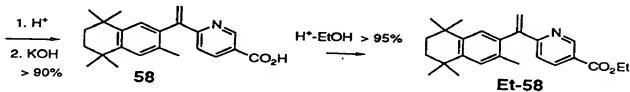
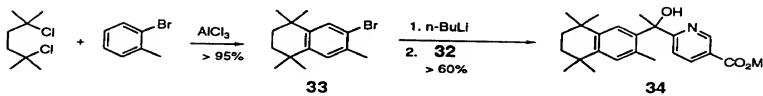
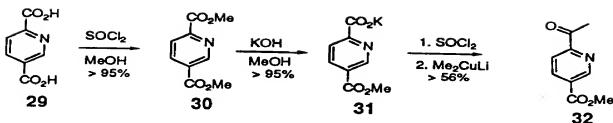
Treatment of compounds 3-methyl-TTNCB and 3-methyl-TTNEB with DCC, *p*-aminophenol, and DMAP resulted in the amino-esters 19 and 20.

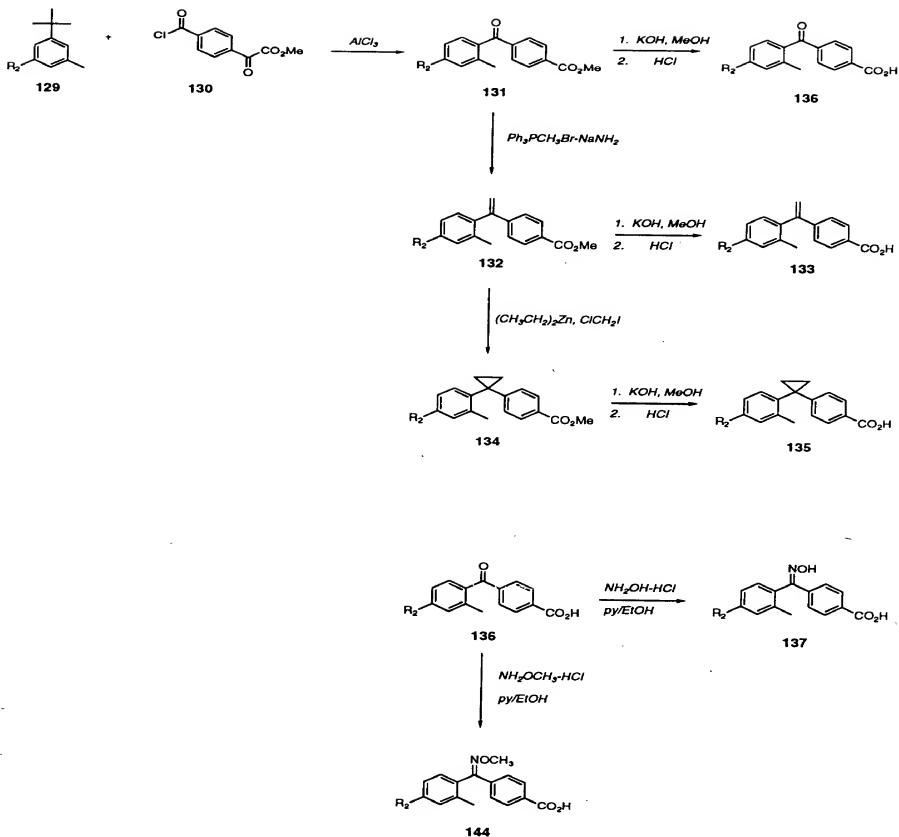


Representative pyridinal derivatives (compounds 21, 23, 26, and 27) may be prepared according to the illustrative synthetic schemes shown above. The synthesis of compound 21 is similar to that previously described for compound 7. Pentamethyl 5 tetrahydronaphthalene 1, pyridinal acid chloride 24, and AlCl₃ are stirred in CH₂Cl₂ to give the ketone 25. Treatment of the ketone 25 with methyl triphosphonium bromide-sodium amide in THF afforded the ethenyl compound 26. Hydrolysis of 26 (KOH, MeOH) followed by acidification gave the acid 21. The cyclopropyl analog 23 was 10 synthesized by treatment of the ethenyl compound 26 with CH₂I₂, zinc dust, CuCl in refluxing ether (Simmons-Smith reaction). Hydrolysis of the resulting cyclopropyl ester 27 was achieved with methanolic KOH followed by acidification to give compound 23. When R₁-R₅ are 15 methyl, for example, compound 62 (TPNCP) is obtained, as shown in Example 33 below.

Other cyclopropyl derivatives such as TPNCB (compound 48) may be likewise prepared by the same method as described for analog 23: olefin 6 is treated with the Simmons-Smith reagent described above, followed by hydrolysis with methanolic-KOH and acidification 20 (HCl) to give the desired cyclopropyl derivative. Epoxy derivatives such as TPNEB (compound 47) may be synthesized by treatment of compound 7 with m-chloroperbenzoic acid at room temperature in CH₂Cl₂ for several hours.

Alternatively, pyridinal analogs, such as compounds 58 25 (TPNEP), 60 (TPNEPC), and 61 (3TTNEPE), may be prepared by the following synthetic route.





Representative derivatives of compounds such as 131-137 and 144 may be prepared according to the illustrative synthetic scheme shown above. Synthesis of such compounds is also described below in examples 44-51.

Illustrative examples for the preparation of some of the compounds according to this invention are as follows:

Example 1

Preparation of compound 3 where R_1 , R_2 , R_3 , R_4 and R_5 are methyl, R' and R'' are oxo, and $X=COOMe$:

To 7 gm (34.7 mmol) of 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene and 6 gm (33.3 mmol) of mono-methyl

teraphthalate in 200 mL of CH_2Cl_2 was added 8 g (38.8 mmol) of PCl_5 .

The reaction boiled vigorously and turned clear within 10 min.

After stirring for an additional 1 h, 6 g (43.5 mmol) of $AlCl_3$ was added in 1 g portions over 15 min. and the reaction was allowed to stir overnight. The mixture was poured into 300 mL of 20% aqueous HCl and extracted with 5% EtOAc-hexanes, dried ($MgSO_4$), concentrated, and crystallized from MeOH to give ca. 6 gm (16.5 mmol) of methyl ester 3.

1H NMR (CD_3OCD_3) δ 1.20 (s, 2(CH_3)), 1.35 (s, 2(CH_3)), 1.75 (s, 2(CH_2)), 2.31 (s, CH_3), 3.93 (s, $COOCH_3$), 7.21 (s, Ar-CH), 7.23 (s, Ar-CH), 7.85 (d, $J=8$ Hz, Ar-2(CH)), 8.18 (d, $J=8$ Hz, Ar-2(CH)).

Example 2

Preparation of compound 4 where R_1 , R_2 , R_3 , R_4 and R_5 are methyl, R' and R'' are oxo, and $X = COOH$ (3-methyl-TTNCB):

To 6 gm (16.5 mmol) of methyl ester 3 suspended in 100 mL of MeOH was added 50 mL of 5N aqueous KOH. The mixture was heated under reflux for 1 h, cooled, acidified (20% aqueous HCl) and the organics extracted with EtOAc. After drying ($MgSO_4$), the product was concentrated and precipitated from 1:4 EtOAc-hexanes to give ca. 5 g (14.3 mmol) of acid 4.

1H NMR (CD_3OCD_3) δ 1.20 (s, 2(CH_3)), 1.35 (s, 2(CH_3)), 1.75 (s, 2(CH_2)), 2.31 (s, CH_3), 7.21 (s, Ar-CH), 7.23 (s, Ar-CH), 7.91 (d, $J=8$ Hz, Ar-2(CH)), 8.21 (d, $J=8$ Hz, Ar-2(CH)).

Example 3

Preparation of compound 5 where R₁, R₂, R₃, R₄ and R₅ are methyl, R' = H and R" = OH, and X = COOH (3-methyl-TTNHMB):

To a 1:1 THF-MeOH solution containing 1 g (2.86 mmol) of ketone 4 was added 100 mg of NaBH₄. The mixture was heated to 50°C for 10 min., cooled, acidified (20% aqueous HCl), and the organics extracted (EtOAc). After drying (MgSO₄), the product was concentrated and precipitated from 1:3 EtOAc-hexanes to give 550 mg (1.56 mmol) of the alcohol 5.

10 ¹HNMR (CD₃OCD₃) δ 1.20 (s, CH₃), 1.22 (s, (CH₃)), 1.22 (s, 2(CH₃)), 1.65 (s, 2(CH₂)), 2.21 (s, CH₃), 6.00 (s, -CHOH-), 7.09 (s, Ar-CH), 7.41 (s, Ar-CH), 7.53 (d, J=8 Hz, Ar-2(CH)), 8.01 (d, J=8 Hz, Ar-2(CH)).

Example 4

15 Preparation of compound 6 where R₁, R₂, R₃, R₄ and R₅ are methyl, R' and R" are methano, and X = COOME:

To 1 gm of methyl ester 3 (2.7 mmol) in 25 mL of dry THF was added 1.2 g (3.08 mmol) of methyltriphosphonium bromide-sodium amide. The solution was stirred at RT for 3 h or until complete by 20 TLC (20% EtOAc-hexanes). Water was added and the organics were extracted with EtOAc, dried (MgSO₄), concentrated and purified by SiO₂ chromatography (5% EtOAc-hexanes) followed by crystallization from MeOH to give 700 mg (1.93 mmol) of methano compound 6.

25 ¹HNMR (CD₃OCD₃) δ 1.22 (s, 2(CH₃)), 1.30 (s, 2(CH₃)), 1.72 (s, 2(CH₂)), 1.95 (s, CH₃), 3.85 (s, COOCH₃), 5.29 (s, =CH), 5.92 (s, =CH), 7.19 (s, Ar-CH), 7.20 (s, Ar-CH), 7.39 (d, J=8 Hz, Ar-2(CH)), 7.96 (d, J=8 Hz, Ar-2(CH)).

Example 5

Preparation of compound 7 where R₁, R₂, R₃, R₄ and R₅ are methyl, R' and R" are methano, and X = COOH (3-methyl-TTNEB):

To 500 mg of methano compound 6 (1.38 mmol) in 20 mL of MeOH was added 5 mL of 5 N aqueous KOH and the suspension was refluxed for 1 h. After acidification (20% aqueous HCl) the organics were extracted (EtOAc), dried (MgSO₄), concentrated, and the solids recrystallized from EtOAc-hexanes 1:5 to give 350 mg (1.0 mmol) of the carboxylic acid 7.

10 ¹H NMR (CD₃OCD₃), δ 1.22 (s, 2(CH₃)), 1.30 (s, 2(CH₃)), 1.72 (s, 2(CH₂)), 1.95 (s, CH₃), 5.22 (s, =CH), 5.89 (s, =CH), 7.19 (s, Ar-CH), 7.20 (s, Ar-CH), 7.39 (d, J=8 Hz, Ar-2(CH)), 7.96 (d, J = 8 Hz, Ar-2(CH)).

Example 6

15 Preparation of compound 37 where R₁, R₂, R₃, R₄ are methyl, R₅ is isopropyl, R' and R" are oxo, and X=COOH (3-IPR-TTNCB):

The compound was prepared in a manner similar to that of compound 4 except that 6-isopropyl-1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1 and 2. MP: 254 °C; ¹H-NMR (CDCl₃) δ 1.19 (d, J=7 Hz, CH(CH₃)₂), 1.21 (s, 2(CH₃)), 1.33 (s, 2(CH₃)), 1.70 (s, 2(CH₂)), 3.12 (q, J=7 Hz, CH(CH₃)₂), 7.14 (s, Ar-CH), 7.37 (s, Ar-CH), 7.92 (d, J=8 Hz, Ar-2(CH)), 8.18 (d, J=8 Hz, Ar-2(CH)).

Example 7

25 Preparation of compound 38 where R₁, R₂, R₃, R₄ are methyl, R₅ is chloro, R' and R" are oxo, and X=COOH (3-chloro-TTNCB):

The compound was prepared in a manner similar to that of compound **4** except that 6-chloro-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1 and 2. MP: 254 °C; ¹H-
5 NMR (CDCl₃) δ1.26 (s, 2(CH₃)), 1.32 (s, 2(CH₃)), 1.72 (s, 2(CH₂)), 7.35
(s, Ar-CH), 7.36 (s, Ar-CH), 7.91 (d, J=8 Hz, Ar-2(CH)), 8.19 (d, J=8
Hz, Ar-2(CH)).

Example 8

Preparation of compound **39** where R₁, R₂, R₃, R₄ are methyl, R₅ is hydroxy, R' and R" are oxo, and X = COOH (**3-hydroxy-TTNCB**):

The compound was prepared in a manner similar to that of compound **4** except that 6-hydroxy-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1 and 2. MP: 264 °C; ¹H-
15 NMR (CDCl₃) δ1.17 (s, 2(CH₃)), 1.31 (s, 2(CH₃)), 1.68 (s, 2(CH₂)), 7.02
(s, Ar-CH), 7.44 (s, Ar-CH), 7.77 (d, J=8 Hz, Ar-2(CH)), 8.27 (d, J=8
Hz, Ar-2(CH)), 11.50 (s, -OH).

Example 9

Preparation of compound **40** where R₁, R₂, R₃, R₄ are methyl, R₅ is ethyl, R' and R" are oxo, and X=COOH (**3-Et-TTNCB**):

The compound was prepared in a manner similar to that of compound **4** except that 6-ethyl-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1 and 2. MP: 226 °C; ¹H-
25 NMR (CDCl₃) δ1.16 (t, J=7.5 Hz, -CH₂CH₃), 1.19 (s, 2(CH₃)), 1.32
(s, 2(CH₃)), 1.69 (s, 2(CH₂)), 2.69 (q, J=7.5 Hz, CH₂CH₃), 7.20 (s, Ar-
CH), 7.25 (s, Ar-CH), 7.87 (brd, Ar-2(CH)), 8.20 (brd, Ar-2(CH)).

Example 10

Preparation of compound 41 where R₁, R₂, R₃, R₄ are methyl, R₅ is bromo, R' and R" are oxo, and X = COOH (3-bromo-TTNCB):

The compound was prepared in a manner similar to that of compound 4 except that 6-bromo-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1 and 2. MP: 275 °C; ¹H-NMR (CDCl₃) δ 1.25 (s, 2(CH₃), 1.32 (s, 2(CH₃)), 1.71 (s, 2(CH₂)), 7.30 (s, Ar-CH), 7.54 (s, Ar-CH), 7.90 (d, J=8 Hz, Ar-2(CH)), 8.18 (d, J=8 Hz, Ar-2(CH)).

Example 11

Preparation of compound 42 where R₁, R₂, R₃, R₄ are methyl, R₅ is isopropyl, R' and R" are methano, and X = COOH (3-IPR-TTNEB):

The compound was prepared in a manner similar to that of compound 7 except that 6-isopropyl-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 252 °C; ¹H-NMR (CDCl₃) δ 1.05 (d, J=7 Hz, CH(CH₃)₂), 1.27 (s, 2(CH₃)), 1.32 (s, 2(CH₃)), 1.70 (s, 2(CH₂)), 2.73 (q, J=7 Hz, CH(CH₃)₂), 5.32 (s, =CH), 5.87 (s, =CH) 7.06 (s, Ar-CH), 7.23 (s, Ar-CH), 7.40 (d, J=8 Hz, Ar-2(CH)), 8.040 (d, J=8 Hz, Ar-2(CH)).

Example 12

Preparation of compound 43 where R₁, R₂, R₃, R₄ are methyl, R₅ is chloro, R' and R" are methano, and X=COOH (3-chloro-TTNEB):

The compound was prepared in a manner similar to that of compound 7 except that 6-chloro-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 233 °C; ¹H-NMR (CDCl₃) δ 1.28 (s, 2(CH₃)), 1.31 (s, 2(CH₃)), 1.71

(s,2(CH₂)), 5.42 (s,=CH), 5.89 (s,=CH), 7.23 (s,Ar-CH), 7.28 (s,Ar-CH), 7.37 (d,J=8 Hz,Ar-2(CH)), 8.03 (d,J=8 Hz,Ar-2(CH)).

Example 13

Preparation of compound 44 where R₁,R₂,R₃,R₄ are methyl, R₅ is hydroxy, R' and R" are methano, and X = COOH (3-hydroxy-TTNEB):

The compound was prepared in a manner similar to that of compound 7 except that 6-hydroxy-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 216 °C; ¹H-NMR (CDCl₃) δ1.21 (s,2(CH₃), 1.30 (s,2(CH₃)), 1.68 (s,2(CH₂)), 5.54 (s,=CH), 5.94 (s,=CH), 6.86 (s,Ar-CH), 7.00 (s,Ar-CH), 7.48 (d,J=8.4 Hz, Ar-2(CH)), 8.07 (d,J=8.4 Hz, Ar-2(CH)).

Example 14

Preparation of compound 45 where R₁,R₂,R₃,R₄ are methyl, R₅ is ethyl, R' and R" are methano, and X = COOH (3-Et-TTNEB):

The compound was prepared in a manner similar to that of compound 7 except that 6-ethyl-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 236 °C; ¹H-NMR (CDCl₃) δ0.99 (t,J=7.6 Hz,-CH₂CH₃), 1.27 (s,2(CH₃)), 1.31 (s,2(CH₃)), 1.70 (s,2(CH₂)), 2.29 (q,J=7.6 Hz,-CH₂CH₃), 5.34 (s,=CH), 5.83 (s,=CH), 7.08 (s,Ar-CH), 7.12 (s,Ar-CH), 7.38 (d,J=8 Hz, Ar-2(CH)), 8.00 (d,J=8 Hz, Ar-2(CH)).

Example 15

Preparation of compound **46** where R_1, R_2, R_3, R_4 are methyl, R_5 is bromo, R' and R'' are methano, and $X = COOH$ (**3-bromo-TTNEB**):

The compound was prepared in a manner similar to that of compound **7** except that 6-bromo-1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 235 °C; 1H -NMR ($CDCl_3$) δ 1.27 (s, $2(CH_3)$), 1.31 (s, $2(CH_3)$), 1.71 (s, CH_3), 5.40 (s, =CH), 5.90 (s, =CH), 7.26 (s, Ar-CH), 7.36 (s, Ar-CH), 7.43 (d, $J=8$ Hz, Ar- $2(CH)$), 8.04 (d, $J=8$ Hz, Ar- $2(CH)$).

Example 16

Preparation of compound **47** where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R'' taken together are CH_2-O (epoxide), and $X = COOH$ (**TPNEB**):

The compound was prepared from compound **6** where R_1, R_2, R_3, R_4, R_5 are methyl. To 1 g (2.76 mmol) of olefin **6** in 5 mL of CH_2Cl_2 was added 600 mg (3.46 mmol) of mCPBA and the reaction was stirred at room temperature for 2 h. Water was added followed by extraction of the organics with ether. The ether layer was washed with water, 1N Na_2CO_3 , brine and dried ($MgSO_4$), filtered and concentrated. Crystallization from MeOH gave the desired epoxide-methyl ester. The methyl ester was hydrolyzed in refluxing methanolic KOH followed by acidification (1N HCl) to give the crude epoxide-acid **47** which was purified by crystallization from EtOAc-hex to give 600 mg (1.64 mmol) of a white powder (59% yield). MP: 168 °C; 1H -NMR ($CDCl_3$) δ 1.26 (s, CH_3), 1.27 (s, CH_3), 1.30 (s, CH_3), 1.31 (s, CH_3), 1.69 (s, $2CH_2$), 2.14 (s, CH_3), 3.15 (d, $J=5.6$ Hz, $CH-O$), 3.41 (d, $J=5.6$ Hz, $CH-O$), 7.09 (s, Ar-CH), 7.28 (d, $J=8.3$ Hz, Ar- $2(CH)$), 7.32 (s, Ar-CH), 8.01 (d, $J=8.3$ Hz, Ar- $2(CH)$).

Example 17

Preparation of compound 48 where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R'' taken together are CH_2-CH_2 (cyclopropyl), and $X = COOH$ (TPNCB):

The compound was prepared from compound 6 where R_1, R_2, R_3, R_4, R_5 are methyl. To a dry 100 mL three necked round bottom flask fitted with a reflux condenser, dropping funnel, and magnetic stir bar was added 722 mg (11.65 mmol) of zinc dust, 109 mg (1.105 mmol) of cuprous chloride ($CuCl$), 7.5 mL of dry THF, and 1.48 g (5.52 mmol) of diiodomethane. To the addition funnel is added 1g (2.76 mmol) of compound 6 in 5 mL of dry THF. The flask is heated to 80 °C, followed by dropwise addition of 6. After the addition of 6 was complete, the reaction was allowed to reflux for 30 h or until completion, followed by dilution with 50 mL of ether and 20 mL of saturated aqueous ammonium chloride solution. The organic layer was washed with 10% NaOH (3 x 20 mL), brine and dried over anhydrous $MgSO_4$. The product was concentrated and purified by preparative TLC (2% EtOAc-hexane) to give 220 mg (0.59 mmol) of the methyl ester of 48. Hydrolysis of the methyl ester with refluxing methanolic KOH, followed by acidification (1N HCl), gave 150 mg (0.41 mmol) of the desired compound 48 after crystallization from EtOAc-hexane (15% yield). MP: 244 °C; 1H -NMR ($CDCl_3$) δ 1.28 (s, 4 (CH_3)), 1.39 (s, CH_2-CH_2), 1.69 (s, 2(CH_2)), 2.12 (s, CH_3), 6.98 (d, $J=8.4$ Hz, Ar-2(CH)), 7.06 (s, Ar-CH), 7.29 (s, Ar-CH), 7.91 (d, $J=8.4$ Hz, Ar-2(CH)).

Example 18

Preparation of compound 49 where R_1, R_2, R_3, R_4, R_5 are methyl, $R' = H$ and $R'' = CH_3$, and $X = COOH$ (PTNEB):

The compound was prepared from compound 7 where R_1, R_2, R_3, R_4, R_5 are methyl. To 1g (2.87 mmol) of compound 7 in 25 mL of EtOAc was added 10 mg of 10% Pd/C. The mixture was degassed under

vacuum followed by addition of H_2 , and allowed to stir under H_2 for 2 h. The reaction was filtered through celite and the product crystallized from EtOAc-hexane to give 750 mg (2.14 mmol) of the desired product **49** (75% yield). MP: 208 °C; 1H -NMR (CDCl₃) δ1.24 (s,CH₃), 1.25 (s,CH₃), 1.26 (s,CH₃), 1.29 (s,CH₃), 1.61 (d,J=7.2 Hz,CH₃), 1.67 (s,2(CH₂)), 2.12 (s,CH₃), 4.30(q,J=7.2 Hz,CH), 7.02 (s,Ar-CH), 7.20 (s,Ar-CH), 7.24 (d,J=8.4 Hz,Ar-2(CH)), 7.99 (d,J=8.4 Hz,Ar-2(CH)).

Example 19

Preparation of compound **50** where R₁, R₂, R₃, R₄, R₅ are methyl, R' and R" = methyldiene cyclopentane, and X = COOH (PTNCB):

The compound was prepared from compound **4** and where R₁, R₂, R₃, R₄, R₅ are methyl. To 1g (2.87 mmol) of **4** in 25 mL of THF at 0 °C was added 8.6 mL of a 1M cyclopentenyl magnesium chloride solution (8.6 mmol). After stirring for 30 m, water was added and acidified with 5 N HCl. The acidified mixture was heated for 5 m, cooled, and the organic product extracted with EtOAc. The EtOAc layer was washed with water and brine, dried (MgSO₄), filtered, and concentrated to give the crude product. Crystallization from EtOAc-hexane gave 340 mg (0.85 mmol) of **50** as a white powder (30% yield). MP: 201 °C; 1H -NMR (CDCl₃) δ1.27 (s,4(CH₃)), 1.64 (br t,CH₂), 1.68 (s,2(CH₂)), 1.70 (br t,CH₂), 1.97 (s,CH₃), 2.15 (br t,CH₂), 2.56 (br t,CH₂), 7.04 (s,Ar-CH), 7.05 (s,Ar-CH), 7.29 (d,J=8 Hz,Ar-2(CH)), 7.97 (d,J=8 Hz,Ar-2(CH)).

Example 20

Preparation of compound **51** where R₁, R₂, R₃, R₄, R₅ are methyl, R' and R" = isopropylidene, and X = COOH (PTNIB):

The compound was prepared from compound **4** and where R₁, R₂, R₃, R₄, R₅ are methyl. To 1g (2.87 mmol) of **4** in 25 mL of THF at

0°C was added 8.6 mL of a 1M isopropyl magnesium chloride solution (8.6 mmol). After stirring for 30 m, water was added and acidified with 5 N HCl. The acidified mixture was heated for 5 m, cooled, and the organic product extracted with EtOAc. The EtOAc layer was
5 washed with water and brine, dried ($MgSO_4$), filtered, and concentrated to give the crude isopropylidene product.
Crystallization from EtOAc-hexane gave 550 mg (1.46 mmol) of 51 as a white powder (51% yield). MP: 297°C; 1H -NMR ($CDCl_3$) δ 1.25 (br s, 4(CH_3)), 1.64 (s, =C CH_3), 1.66 (s, =C CH_3), 1.87 (s, 2(CH_2)), 1.96
10 (s, CH_3), 7.00 (s, Ar-CH), 7.03 (s, Ar-CH), 7.25 (d, J =8 Hz, Ar-2(CH)), 7.97 (d, J =8 Hz, Ar-2(CH)).

Example 21

Preparation of compound 52 where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R'' = oxo, Z = S, and X = COOH (TTNCTC):

15 To 1 g (4.9 mmol) of 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydro-naphthalene and 1 g (4.9 mmol) of mono methyl thiophene carboxylic acid chloride in 25 mL of CH_2Cl_2 was added 1 g (7.5 mmol) of $AlCl_3$. The reaction was heated to reflux for 15 m followed by cooling and addition of 20% aqueous HCl. The product was extracted
20 with EtOAc, washed (H_2O , brine), dried ($MgSO_4$), filtered, concentrated, and purified by crystallization from MeOH to give 450 mg (1.21 mmol) of the methyl ester of 52 (25% yield). The methyl ester was hydrolyzed in methanolic KOH followed by acidification (20% HCl) extraction with EtOAc, washed (H_2O , brine), dried ($MgSO_4$),
25 filtered, concentrated, and purified by crystallization from EtOAc-hexane to give 375 mg (1.05 mmol) of 52 (87% yield). MP: 206 °C; 1H -NMR ($CDCl_3$) δ 1.26 (s, 2(CH_3)), 1.31 (s, 2(CH_3)), 1.71 (s, 2(CH_2)), 2.38 (s, CH_3), 7.21 (s, Ar-CH), 7.44 (s, Ar-CH), 7.48 (d, J =4 Hz, Thio Ar-CH), 7.85 (d, J =4 Hz, Thio Ar-CH).

Example 22

Preparation of compound 53 where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R'' = methano, $Z = S$, and $X = COOH$ (TTNETC):

Compound 53 was prepared from the methyl ester of 52 in a manner similar to examples 4 and 5. MP: 200 °C; 1H -NMR ($CDCl_3$) δ 1.26 (s, $2(CH_3)$), 1.30 (s, $2(CH_3)$), 1.69 (s, $2(CH_2)$), 2.10 (s, CH_3), 5.21 (s, =CH), 5.88 (s, =CH), 6.76 (d, $J=4$ Hz, Thio Ar-CH), 7.11 (s, Ar-CH), 7.23 (s, Ar-CH), 7.68 (d, $J=4$ Hz, Thio Ar-CH).

Example 23

Preparation of compound 54 where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R'' = oxo, and $X =$ tetrazole (3-methyl-TTNCBT):

To 500 mg (1.51 mmol) of 4-[(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)carbonyl]benzonitrile (synthesized by $AlCl_3$ catalyzed condensation of 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene with 4-cyanobenzoic acid chloride in CH_2Cl_2) in toluene was added 342 mg (1.66 mmol) of trimethyl tin azide. The mixture was refluxed for 23 h and cooled to give 537 mg (1.44 mmol) of the desired tetrazole 54 as a white precipitate (96% yield). LRMS: 374.15; 1H -NMR (CD_3SOCD_3) δ 1.19 (s, $2(CH_3)$), 1.32 (s, $2(CH_3)$), 1.70 (s, $2(CH_2)$), 2.25 (s, CH_3), 3.19 (s, N-H), 7.30 (s, Ar-CH), 7.32 (s, Ar-CH), 7.90 (d, $J=8$ Hz, Ar-2(CH)), 8.20 (d, $J=8$ Hz, Ar-2(CH)).

Example 24

Preparation of compound 55 where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R'' = methano, and $X =$ tetrazole (3-methyl-TTNEBT):

To 500 mg (1.52 mmol) of 4-[(1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzonitrile (synthesized by $AlCl_3$ catalyzed condensation of 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene with 4-cyanobenzoic acid chloride in CH_2Cl_2 followed by treatment of the ketone with $CH_3PPh_3Br-NaNH_2$) in toluene

was added 342 mg (1.67 mmol) of trimethyl tin azide. The mixture was refluxed for 23 h and cooled to give 535 mg (1.44 mmol) of the desired tetrazole 55 as a white precipitate (95% yield). LRMS: 372.25; $^1\text{H-NMR}$ (CD_3SOCD_3) δ 1.21 (s, 2(CH_3)), 1.24 (s, 2(CH_3)), 1.68 (s, 2(CH_2)), 1.92 (s, CH_3), 2.55 (s, N-H), 5.27 (=CH), 5.97 (s, =CH), 7.10 (s, Ar-CH), 7.18 (s, Ar-CH), 7.47 (d, $J=8$ Hz, Ar-2(CH)), 8.00 (d, $J=8$ Hz, Ar-2(CH)).

Example 25

Preparation of compound 25 where $\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4$ are methyl, R' and R'' = 10 oxo, and $\text{X} = \text{COOMe}$:

The compound was prepared in a manner similar to that of compound 4 except that 1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene and 4-methyl ester pyridinic 2-acid chloride was substituted for mono-methyl terephthalic acid chloride (see examples 1 and 2).

Example 26

Preparation of compound 56 where $\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4$ are methyl, R' and R'' = methano, and $\text{X} = \text{COOH}$ (TTNEP):

Compound 25 was treated with $\text{CH}_3\text{PPh}_3\text{Br-NaNH}_2$ as in example #4. Hydrolysis of the resulting olefinic methyl ester with methanolic KOH, followed by acidification (20% HCl) and crystallization from EtOAc-hexane gave compound 56. MP: 173 °C; $^1\text{H-NMR}$ (CDCl_3) δ 1.26 (s, (CH_3)), 1.27 (s, CH_3), 1.30 (s, 2(CH_3)), 1.70 (s, (CH_2)), 5.70 (s, =CH), 6.10 (s, =CH), 7.08 (d, $J=8$ Hz, Pyr-CH), 7.27 (s, Ar-CH), 7.19 (d, $J=8$ Hz, Ar-CH), 7.39 (d, $J=8$ Hz, Ar-CH), 8.28 (d, $J=8$ Hz, Pyr-CH), 9.31 (s, Pyr-CH).

Example 27

Preparation of compound 57 where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R'' = oxo, and X = COOH:

Compound 57 was prepared in a manner similar to that of compound 6 (example #4) except that 4-methylester-pyridinic-2-acid chloride was substituted for mono-methyl terephthalic acid chloride (see examples 1 and 2). The resulting methyl ester was hydrolyzed as in example #5 to give compound 57. $^1\text{H-NMR}$ (CDCl_3) δ 1.22 (s, 2(CH_3)), 1.30 (s, 2(CH_3)), 1.69 (s, 2(CH_2)), 2.40 (s, CH_3), 7.22 (s, Ar-CH), 7.43 (s, Ar-CH), 8.13 (d, $J=8.0$ Hz, Pyr-CH), 8.54 (d, $J=8$ Hz, Pyr-CH), 9.34 (s, Pyr-CH).

Example 28

Preparation of compound 58 where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R'' = methano, and X = COOH (TPNEP):

The methyl ester from example #26 was treated with $\text{CH}_3\text{PPh}_3\text{Br-NaNH}_2$ as in example #4 followed by hydrolysis with methanolic KOH at reflux for 1 h and acidification with 20% aqueous HCl and crystallization from EtOAc-hexane to give compound 58. MP: 235 °C; $^1\text{H-NMR}$ (CDCl_3) δ 1.27 (s, 2(CH_2)), 1.31 (s, 2(CH_3)), 1.70 (s, 2(CH_2)), 2.00 (s, CH_3), 5.55 (s, =CH), 6.57 (s, =CH), 7.06 (d, $J=8.3$ Hz, Pyr-CH), 7.12 (s, Ar-CH), 7.14 (s, Ar-CH), 8.20 (d, $J=8.1$ Hz, Pyr-CH). 9.29 (s, Pyr-CH).

Example 29

Preparation of methyl 2-acetyl-5-pyridinecarboxylate 32:

To a slurry of the 2,5-pyridinedicarboxylic acid 29 (34 g, 0.2 mol) in 120 mL of methanol at 0°C was added dropwise 15 mL of thionyl chloride and the resulting slurry was warmed up to room temperature, giving rise to a clear solution. The mixture then was heated at reflux for 12h and to afford a yellow slurry. Filtration

of the reaction mixture provided dimethyl-2,5-pyridinedicarboxylate 30 in quantitative yield as a yellow crystalline solid.

5 The pyridinedicarboxylate 30 (19.5 g, 0.1 mol) was treated with solid KOH (6.51 g, 0.1 mol) in 300 mL of methanol at room temperature for 2 h, giving rise to a thick pale white suspension, which was filtered and dried to provide the mono-potassium pyridinecarboxylate 3 in quantitative yield.

10 The crude mono-pyridinecarboxylate 31 (880 mg, 4 mmol) was treated with 3 mL of thionyl chloride at reflux for 2h and the excess SOCl_2 was removed by the usual method. To the crude acid chloride in 8 mL of THF at -78°C was added slowly a freshly prepared 1.0M ether solution (5.5 mL, 5.5 mmol) Me_2CuLi . The resulting dark slurry was allowed to stir at -78°C for 60 min. and then was quenched with 2% HCl. Standard work-up and chromatography 15 of the crude mixture afforded methyl-2-acetyl-5-pyridinecarboxylate 32 in over 56% yield as a yellow solid.

Example 30

Preparation of compound 58 (TPNEP) (by an alternate scheme than in Example 28) and of corresponding ester Et-58 where $\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4, \text{R}_5$ are 20 methyl, R' and R'' are methano, and $= \text{COOH}$:

25 A solution of 2-bromotoluene (8.5 g, 50 mmol) and 2,2-dichloro-2,2-dimethylhexane (9.15 g, 50 mmol) in 100 mL of dichloroethane was treated with aluminum trichloride (0.66 g, 5 mmol). The resulting dark brown solution was allowed to stir at room temperature for 30 min. and was then quenched with ice. Removal of solvent and recrystallization from methanol afforded 2-bromo-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene 33 in 95% yield as a white solid. A THF (4 mL) solution containing the bromocompound 33 (141 mg, 0.5 mmol) at -78°C was treated with a 30 1.6 M hexane solution (0.4 mL, 0.6 mmol) of n-BuLi, and the

resulting mixture was then cannulated to a THF (2 mL) solution of the 2-acetyl-5-pyridinecarboxylate **32** (72 mg, 0.4 mmol) at -78 °C. The mixture was allowed to stir at -78 °C for 60 min. and was quenched with 2% HCl. Removal of the solvent and chromatography of the crude mixture provided the intermediate **34**, which was then treated with 5% HCl at reflux followed by KOH-MeOH at 70 °C for 30 min. Standard work-up and chromatography of the crude mixture provided 2-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl] pyridine-5-carboxylic acid **58** in over 50% yield as a white solid. ¹H-NMR (CDCl₃) δ 1.27 (s, 2(CH₃)), 1.31 (s, 2(CH₃)), 1.70 (s, 2(CH₂)), 2.00 (s, CH₃), 5.56 (s, =CH), 6.55 (s, =CH), 7.08 (d, J = 8.3 Hz, Pyr-CH), 7.12 (s, Ar-CH), 7.15 (s, Ar-CH), 8.23 (d, J = 8.3 Hz, Pyr-CH) and 9.32 (s, Pyr-CH).

Treatment of the pyridinecarboxylic acid **58** (15 mg, 0.004 mmol) with one drop of SOCl₂ in 5 mL of ethanol at reflux for 60 m, followed by a flash chromatography, gave rise to a quantitative yield of the ethyl ester Et-**58** as a white solid. ¹H-NMR (CDCl₃) δ 1.27 (s, 2(CH₃)), 1.31 (s, 2(CH₃)), 1.40 (t, J = 7.1 Hz, -CH₂CH₃), 1.70 (s, 2(CH₂)), 1.99 (s, CH₃), 4.40 (q, J = 7.1 Hz, -CH₂CH₃), 5.51 (s, =CH), 6.53 (s, =CH), 7.01 (d, J = 8.0 Hz, Pyr-CH), 7.12 (s, Ar-CH), 7.14 (s, Ar-CH), 8.15 (d, J = 8.0 Hz, Pyr-CH) and 9.23 (s, Pyr-CH).

Example 31

Preparation of 3-acetyl-2-pyridinecarboxylic acid N,N-diisopropylamide **36a**:

The mono-potassium pyridinecarboxylate **31** (1.1 g, 5 mmol) was treated with SOCl₂ (5 mL, excess) at 70 °C for 2h and the excess thionyl chloride was removed to give a yellowish solid. To a solution of diisopropylamine (1 g, 10 mmol) in 10 mL of methylene chloride at 0 °C was added the CH₂Cl₂ solution (10 mL) of the above

acid chloride. The resulting slurry was allowed to stir at room temperature for 3 h and was filtered from the ammonium salts.

Removal of solvent and chromatography of the crude residue afforded the product **36a** in 90% yield as a white solid.

5 Example 32

Preparation of compounds **60** (TPNEPC) and **61** (3TTNEPE):

2-Bromo-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene **33** (620 mg, 2.2 mmol) and the acetylpyridineamide **36a** (500 mg, 2 mmol) were converted by a similar method as described above to obtain the intermediate **34** in over 80% yield. To a solution of the pyridine amide **34** (432 mg, 1 mmol) in 5 mL of THF at -78°C was added 1.5 M DIBAL toluene solution (0.7 mL, 1.05 mmol) and the resulting yellow clear solution was warmed up to -20 °C slowly in 60 min. and then was quenched with water. Removal of solvent and chromatography of the crude mixture afforded the pyridinealdehyde **35** in 83% yield as a white solid. ¹H-NMR (CDCl₃) δ1.25 (s,2(CH₃)), 1.29 (s,2(CH₃)), 1.70 (s,2(CH₂)), 1.96 (s,CH₃), 5.47 (s,=CH), 5.92 (s,=CH), 7.10 (s,Ar-CH), 7.11 (s,Ar-CH), 7.70 (d,J = 8.0 Hz, Pyr-CH), 7.88 (d,J = 8.0 Hz, Pyr-CH), 8.72 (s, Pyr-CH), 10.06 (s,CHO).

The pyridinealdehyde **35** (10 mg, 0.03 mmol) was treated with 2.0 mL of H₂O₂ in 2 mL of methanol-watér 1:1 mixture at room temperature for 10 h and then was quenched with 10% HCl. Extraction of the mixture with EtOAc (40 mL) and removal of solvent gave rise to 5-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]pyridine-2-carboxylic acid **60** as a white solid in almost quantitative yield. ¹H-NMR (CDCl₃) δ1.26 (s,2(CH₃)), 1.30 (s,2(CH₃)), 1.70 (s,2(CH₂)), 1.94 (s,CH₃), 5.47 (s,=CH), 5.92 (s,=CH), 7.10 (s,Ar-2(CH)), 7.76 (bs, Pyr-CH, 8.16 (bs, Pyr-CH) and 8.65 (s, Pyr-CH).

Treatment of the pyridinecarboxylic acid **60** (5 mg) with one drop of SOCl_2 in 1 mL of ethanol at reflux for 60 min followed by a flash chromatography gave rise to a quantitative yield of the ethyl ester **61** as a white solid. $^1\text{H-NMR}$ (CDCl_3) δ 1.26 (s, $2(\text{CH}_3)$), 1.29 (s, $2(\text{CH}_3)$), 1.44 (t, $J = 7.1$ Hz, $-\text{CH}_2\text{CH}_3$), 1.69 (s, $2(\text{CH}_2)$), 1.95 (s, CH_3), 4.46 (q, $J = 7.1$ Hz, $-\text{CH}_2\text{CH}_3$), 5.43 (s, =CH), 5.88 (s, =CH), 7.09 (s, Ar-CH), 7.10 (s, Ar-CH), 7.64 (d, $J = 8.0$ Hz, Pyr-CH), 8.03 (d, $J = 8.0$ Hz, Pyr-CH) and 8.68 (s, Pyr-CH).

Example 33

Preparation of compound **62** where $\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4, \text{R}_5$ are methyl and R' and R'' together are CH_2CH_2 (TPNCP):

To 162 mg (2.48 mmol) of zinc dust, 25 mg (0.25 mmol) of CuCl , and 332 mg (1.24 mmol) of CH_2I_2 in 3 mL of dry ether was added dropwise 150 mg (0.413 mmol) of olefin **26** where $\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4, \text{R}_5$ are methyl in 5 mL of dry ether. The mixture was heated at reflux for 12 h or until complete by H-NMR . Water was added and the organics extracted with ether, washed with NH_4Cl , brine and dried over MgSO_4 . The desired cyclopropyl compound was purified by crystallization from ether-MeOH to give 60 mg (0.159 mmol) of the methyl ester of **62** as a pale yellow solid (39% yield). MP: 177 °C; $^1\text{H-NMR}$ (CDCl_3) δ 1.27 (s, $2(\text{CH}_3)$), 1.31 (s, $2(\text{CH}_3)$), 1.35 (s, CH_2), 1.70 (s, $2(\text{CH}_2)$), 1.85 (s, CH_2), 2.11 (s, CH_3), 3.90 (s, CH_3), 6.75 (d, $J = 8.0$ Hz, Pyr-CH), 7.14 (s, Ar-CH), 7.26 (s, Ar-CH), 7.98 (d, $J = 8.0$ Hz, Pyr-CH) and 9.23 (s, Pyr-CH).

To 60 mg (0.16 mmol) of the above methyl ester in 10 mL of MeOH was added 1 mL of an aqueous 6N KOH solution. After stirring at room temperature for 1 h, the hydrolysis was complete and the reaction was acidified with 1 N aqueous HCl until the solids precipitated. The product was extracted with ether, washed with water, brine and dried over MgSO_4 . Crystallization from EtOAc-

hexanes gave 33 mg (0.094 mmol) of the pyridinal carboxylic acid **62** (59% yield). MP: 275 °C; ¹H-NMR(CDCl₃) δ1.25 (s,2(CH₃)), 1.35 (s,2(CH₃)), 1.40 (s,CH₂), 1.72 (s,2(CH₂)), 1.85 (s,CH₂), 2.15 (s,CH₃), 6.78 (d,J = 8.0 Hz, Pyr-CH), 7.14 (s,Ar-CH), 7.26 (s,Ar-CH), 8.02 (d,J = 8.0 Hz, Pyr-CH) and 9.15 (s,Pyridine-CH).

Example 34

Preparation of compound **63** where R₁,R₂,R₃,R₄,R₅ are methyl, R' and R" are methano, X = CONHR₉, and R₉ = 4-hydroxyphenyl (3-methyl-TTNEHBP):

To 750 mg (10 mmol) of DMF in 22 mL of anhydrous ether was added 1.3g (10 mmol) of oxalyl chloride. The reaction was stirred for 1 h, followed by removal of solvent to give a crude white solid (dimethylchloroformadinium chloride). To the dimethylchloroformadinium chloride was added 2.87 g (8.24 mmol) of compound **7** in 12 mL of dry DMF. The reaction was stirred for 20 m at room temperature followed by cooling to 0°C. The cooled solution of the acid chloride of **7** was added dropwise to a cooled DMF (0°C) solution containing 3.62 g (33 mmol) of 4-aminophenol and 1.68 g (16.3 mmol) of triethyl amine. After stirring at 0°C for 30 m, the reaction was warmed to room temperature for 12 h. Aqueous 20% HCl was added and the resulting solid was filtered and washed with water, acetone, and EtOAc to give 600 mg (1.36 mmol) of the desired compound **63** (17% yield). ¹H-NMR (CDCl₃) δ1.29 (s,2(CH₃)), 1.31 (s,2(CH₃)), 1.71 (s,(CH₂)), 1.99 (s,CH₃), 5.31 (s,=CH), 5.80 (s,=CH), 6.85 (d,Ar-2(CH)), 7.09 (s,Ar-CH), 7.16 (s,Ar-CH), 7.40 (d,Ar-2(CH)), 7.48 (d,Ar-2(CH)), 8.40 (d,Ar-2(CH)).

Example 35

Preparation of compound **64** where R₁,R₂,R₃,R₄,R₅ are methyl, R' and R" are methano, X = CONHR₉, and R₉ = 4-fluorophenyl (3-methyl-TTNEFBP):

The compound was prepared in a manner similar to that of compound **63** except that 4-fluoroaniline was substituted for 4-aminophenol. MP: 203°C; ¹H-NMR (CDCl₃) δ1.28 (s,2(CH₃)), 1.31 (s,2(CH₃)), 1.70 (s,2(CH₂)), 1.96 (s,CH₃), 5.33 (s,=CH), 5.81 (s,=CH), 7.05 (d,J=9 Hz,Ar-2(CH)), 7.09 (s,Ar-CH), 7.13 (s,Ar-CH), 7.39 (d,J=8.4 Hz,Ar-2(CH)), 7.59 (dd,J=5,9 Hz,Ar-2CH), 7.75 (brs NH), 7.78 (d,J=8.4 Hz,Ar-2(CH)).

Example 36

Preparation of compound **65** where R₁,R₂,R₃,R₄,R₅ are methyl, R' and R" are methano, X = CONHR₉, and R₉ = 4-phenylcarboxylic acid (**3-methyl-TTNECBP**):

The compound was prepared in a manner similar to that of compound **63** except that methyl 4-aminophenyl carboxylate was substituted for 4-aminophenol. The resulting ester was hydrolyzed in methanolic KOH, followed by acidification (20% HCl) to give the desired compound **65**. MP: 200 °C; ¹H-NMR (CDCl₃) δ1.28 (s,2(CH₃)), 1.31 (s,2(CH₃)), 1.71 (s,2(CH₂)), 1.97 (s,CH₃), 5.34 (s,=CH), 5.85 (s,=CH), 7.09 (s,Ar-CH), 7.14 (s,Ar-CH), 7.40 (d,J=8 Hz,Ar-CH), 7.80 (d,J=8 Hz,Ar-2(CH)), 7.87 (br s,Ar-2(CH)), 8.14 (br s,Ar-2(CH)).

Example 37

Preparation of compound **66** where R₁,R₂,R₃,R₄,R₅ are methyl, R' and R" are oxo, X = CONHR₉, and R₉ = 3-hydroxyphenyl (**3-methyl-m-TTNCHBP**):

To 750 mg (10 mmol) of DMF in 22 mL of anhydrous ether was added 1.3g (10 mmol) of oxalyl chloride. The reaction was stirred for 1 h, followed by removal of solvent to give a crude white solid (dimethylchloroformadinium chloride). To the dimethylchloroformadinium chloride was added 2.88 g (8.24 mmol) of compound **4** in 12 mL of dry DMF. The reaction was stirred for 20 m at room

temperature, followed by cooling to 0°C. The cooled solution of the acid chloride of 7 was added dropwise to a cooled DMF (0°C) solution containing 3.62 g (33 mmol) of 4-aminophenol and 1.68 g (16.3 mmol) of triethyl amine. After stirring at 0°C for 30 m, the 5 reaction was warmed to room temperature for 12 h. Aqueous 20% HCl was added and the resulting solid was filtered and washed with water, acetone, and EtOAc to give 750 mg (1.70 mmol) of the desired compound 66 (21% yield). MP: 182°C; ¹H-NMR (CDCl₃) δ1.22
10 (s,2(CH₃)), 1.32 (s,2(CH₃)), 1.70 (s,2(CH₂)), 2.37 (s,CH₃), 6.58 (m,Ar-2(CH)), 7.20 (d,J=8 Hz,Ar-CH), 7.22 (s,Ar-CH), 7.28 (s,Ar-
Ch), 7.91 (d,J=8.3 Hz,Ar-2(CH)), 8.26 (d,J=8.3 Hz,Ar-2(CH)).

Example 38

Preparation of compound 67 where R₁, R₂, R₃, R₄, R₅ are methyl, R' and R" are methano, X = CONHR₉, and R₉ = 3-hydroxyphenyl (3-methyl-m-
15 TTNEHBP):

The compound was prepared in a manner similar to that of compound 63 except that 3-aminophenol was substituted for 4-aminophenol. MP: 136°C; ¹H-NMR (CDCl₃) δ1.28 (s,2(CH₃)), 1.31 (s,2(CH₃)), 1.70 (s,2(CH₂)), 1.97 (s,CH₃), 5.35 (s,=CH), 5.84
20 (s,=CH), 6.57 (m,Ar-2(CH)), 7.09 (s,Ar-CH), 7.14 (s,Ar-CH), 7.16 (m,Ar-CH), 7.39 (d,J=8.3 Hz,Ar-2(CH)), 8.09 (d,J=8.3 Hz,Ar-2(CH)).

Example 39

Preparation of compound 68 where R₁, R₂, R₃, R₄, R₅ are methyl, R' and R" are methano, X = CONHR₉, and R₉ = 2-hydroxyphenyl (3-methyl-o-
25 TTNCHBP):

The compound was prepared in a manner similar to that of compound 63 except that 2-aminophenol was substituted for 4-aminophenol. MP: 180°C; ¹H-NMR (CDCl₃) δ1.28 (s,2(CH₃)), 1.31 (s,2(CH₃)), 1.71 (s,2(CH₂)), 1.97 (s,CH₃), 5.35 (s,=CH), 5.84

(s,=CH), 6.9 (m,Ar-CH), 7.08-7.2 (m,Ar-CH), 7.09 (s,Ar-CH), 7.13 (s,Ar-CH), 7.42 (d,J=8.4 Hz,Ar-2(CH)), 7.83 (d,J=8.4 Hz,Ar-2(CH)), 8.03 (brs,Ar-CH), 8.64 (s,NH).

Example 40

5 Preparation of compound 69 where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R'' are methano, $X = CONHR_9$, and $R_9 = 3$ -phenylcarboxylic acid (3-methyl-m-TTNECBP):

The compound was prepared in a manner similar to that of compound 63 except that methyl-3-amino phenyl carboxylate was 10 substituted for 4-aminophenol. The resulting ester was hydrolyzed in methanolic KOH followed by acidification (20% HCl) to give the desired compound 69. MP: 250°C; 1H -NMR ($CDCl_3$) δ 1.28 (s,2(CH_3)), 1.31 (s,2(CH_3)), 1.71 (s,2(CH_2)), 1.97 (s, CH_3), 5.34 (s,=CH), 5.85 (s,=CH), 7.09 (s,Ar-CH), 7.14 (s,Ar-CH), 7.40 (d,J=8 Hz, Ar-2(CH)), 15 7.55 (m,Ar-CH), 7.76 (m,Ar-CH), 7.80 (d,J=8 Hz, Ar-2(CH)), 7.87 (s,Ar-CH), 8.14 (s,NH).

Example 41

Preparation of compound 70 where R_1, R_2, R_3, R_4, R_5 are methyl, R' and R'' are methano, $n=0$, and $X = COOH$:

20 The compound was prepared in a manner similar to that of compound 7 except that 1,1,3,3,5-pentamethylindane was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 145°C; 1H -NMR ($CDCl_3$) δ 1.05 (s,2(CH_3)), 1.28 (s, CH_3), 1.31 (s, CH_3), 1.38 (s, CH_2), 1.98 (s, CH_3), 5.34 (s,CH), 5.84 (s,CH), 6.90 (s, Ar-CH), 6.92 (s, Ar-CH), 7.36 (d,J=8.4 Hz, Ar-2(CH)), 25 8.00 (d,J=8.4 Hz, Ar-2(CH)).

Example 42

Preparation of compound **71** where $R_1, R_2, R_3, R_4, R_5, R_{14}$ are methyl, R' and R'' are methano, $n=0$, and $X = COOH$:

The compound was prepared in a manner similar to that of compound **7** except that 1,1,2,3,3,5-pentamethylindane was substituted for 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene in examples 1, 2, 4, and 5. MP: 217°C; 1H -NMR (CDCl₃) 61.01 (d, $J=7.3$ Hz, CH₃), 1.08 (s, CH₃), 1.10 (s, CH₃), 1.27 (s, CH₃), 1.30 (s, CH₃), 1.88 (q, CH), 2.00 (s, CH₃), 5.35 (s, =CH), 5.85 (s, =CH), 6.95 (s, Ar-CH), 6.98 (s, Ar-CH), 7.38 (d, $J=8.3$ Hz, Ar-2(CH)), 8.00 (d, $J=8.3$ Hz, Ar-2(CH)).

Example 43

Preparation of compound **72** where R_1, R_2, R_3, R_4, R_5 , are methyl, R' and R'' are H, and $X = COOH$:

The compound was prepared in a manner similar to that of compound **4** (examples 1 and 2) except that methyl-4-(bromomethyl)benzoate was substituted for mono-methyl terephthalic acid chloride. MP: 237°C; 1H -NMR (CDCl₃) 61.23 (s, 2(CH₃)), 1.27 (s, 2(CH₃)), 1.67 (s, 2(CH₂)), 2.16 (s, CH₃), 4.06 (s, CH₂), 7.01 (s, Ar-CH), 7.08 (s, Ar-CH), 7.25 (d, $J=8$ Hz, Ar-2(CH)), 8.01 (d, $J=8$ Hz, Ar-2(CH)).

Example 44

Preparation of compound **131**:

To 5 gm (24.5 mM) of 3,5-di-tert-butyltoluene **129** and 4.4 gm (22.0 mM) of terephthalic acid monomethyl ester chloride **130** in 50 ml of CH₂Cl₂ was added 6.5 gm (48.9 mM) of AlCl₃ in several portions. The reaction was allowed to stir for 1 h. the mixture was then poured into 100 ml of 20% aqueous HCl and extracted with EtoAc, dried (MgSO₄), concentrated, purified by SiO₂ chromatography

(5% EtOAC-hexanes) to give 4.1 gm (13.2 mM) of methyl ester **131**.

¹H-NMR(CDCl₃): δ1.37 (s, 3(CH₃)), 2.4 (s, CH₃), 3.94 (s, COOCH₃), 7.20-7.32 (m, Ar-3(CH)), 7.85 (d, Ar-2(CH)), 8.13 (d, Ar-2(CH)).

Example 45

5 Preparation of compound **132**:

To 2 gm (6.5 mM) of methyl ester **131** in 40 ml of dry THF was added 3.8 gm (9.7 mM) of methyltriphenylphosphonium bromide-sodium amide. The solution was stirred at RT for 2.5 h. Water was added and the organics were extracted with EtOAC, dried (MgSO₄), 10 concentrated and purified by SiO₂ chromatography (3% EtOAC-hexanes) followed by crystallization from MeOH to give 1.4 gm (4.6 mM) of methano compound **132**. ¹H-NMR(CDCl₃) δ1.30 (s, 3(CH₃)), 2.09 (s, CH₃), 3.89 (s, COOCH₃), 5.29 (s, =CH₂), 5.82 (s, =CH₂), 7.11 (d, Ar-2(CH)), 7.19 (s, Ar-CH), 7.24 (d, Ar-2(CH)), 7.32 (d, Ar-2(CH)), 15 7.96 (d, Ar-2(CH)).

Example 46

Preparation of compound **133**:

To 100 mg (0.32 mM) of methano compound **132** in 10 ml of MeOH was added 2.5 ml of 5N aqueous KOH and the suspension was 20 refluxed for 30 m. After acidification (20% aqueous HCl) the organics were extracted (EtOAC), dried (MgSO₄), concentrated, and the solids recrystallized from EtOAC-hexanes (1:5) to give 91 mg (0.31 mM) of carboxylic acid **133**. MP:202-203°C. ¹H-NMR(CDCl₃) δ1.35 (s, 3(CH)), 2.02 (s, CH₃), 5.34 (s, =CH₂), 5.85 (s, =CH₂), 7.16 (d, Ar-2(CH)), 7.20 (s, Ar-CH), 7.23 (d, Ar-2(CH)), 7.36(d, Ar-2(CH)), 8.0 (d, Ar-2(CH)).

Example 47

Preparation of compound **134**:

In a 50 ml flask, 200 mg (0.65 mM) of olefin 132 in 10 ml of dichloroethane was cooled to 0°C, and 401 mg (3.25 mM) of Et₂Zn was added via syringe. To this solution was added dropwise 1.15 g (6.5 mM) of ClCH₂I. The solution was stirred for 20 m at 0°C, 5 allowed to warm to room temperature, and was stirred for an additional 30 m. The mixture was then heated at 72°C for 10 h. After cooling to 0°C, NH₄Cl was added and the organics extracted with ether, washed with water, brine and dried over MgSO₄. The desired cyclopropyl compound was purified by crystallization from 10 ether-MeOH (1:5) to give 50 mg (0.16 mM) of the methyl ester of 134. ¹H-NMR(CDCl₃): δ1.28 (s, 3(CH₃)), 1.37 (s, CH₂), 1.39 (s, CH₂), 2.18 (s, CH₃), 3.87 (s, COOCH₃), 6.96 (d, Ar-2(CH)), 7.18 (s, Ar-CH), 7.22 (d, Ar-2(CH)), 7.29 (d, Ar-2(CH)), 7.85 (d, Ar-2(CH)).

Example 48

15 **Preparation of compound 135:**

To 15 mg (0.047 mM) of compound 134 in 5 ml of MeOH was added 1 ml of aqueous 5N KOH solution and the suspension was refluxed for 20 m. After acidification (20% aqueous HCl), the organics were extracted (EtOAC), dried (MgSO₄), concentrated, and 20 the solids recrystallized from EtOAC-hexanes 1:4 to give 11 mg (0.036 mM) of the carboxylic acid 135. MP:214-215°C. ¹H-NMR(CDCl₃): δ1.27 (d, CH₂), 1.33 (s, 3(CH₃)), 1.40 (d, CH₂), 2.19 (s, CH₃), 6.99 (d, Ar-2(CH)), 7.18 (s, Ar-CH), 7.20 (d, Ar-2(CH)), 7.29 (d, Ar-2(CH)), 7.90 (d, Ar-2(CH)).

25 **Example 49**

Preparation of compound 136:

Compound 136 was hydrolyzed in a manner similar to that of compound 133 except that compound 131 was substituted for

compound 132. $^1\text{H-NMR}$ (CDCl_3) δ 1.36 (s, 3(CH_3)), 2.41 (s, CH_3), 7.28-7.33 (m, Ar-3(CH)), 7.87 (d, Ar-2(CH)), 8.18 (d, Ar-2(CH)).

Example 50

Preparation of compound 137:

5 To 100 mg (0.34 mM) of compound 136 in 4 ml of EtOH was added 117 mg (1.69 mM) of $\text{NH}_2\text{OH}\cdot\text{HCl}$ and 0.41 ml (5.07 mM) of pyridine. The solution was refluxed for 16 h. 1N HCl was added at RT and the organics extracted with EtOAc, washed with water, brine, dried over MgSO_4 . The oxime compound was purified by
10 crystallization from EtOAc-hexanes (1:5) to give 68 mg (0.22 mM) of a mixture of *trans* and *cis* oximes (2:1) 137. $^1\text{H-NMR}$ (CDCl_3) (*Trans*) δ 1.36 (s, 3(CH_3)), 2.18 (s, CH_3), 7.06 (d, Ar-2(CH)), 7.20 (d, Ar-2(CH)), 7.33 (s, Ar- CH), 7.57 (d, Ar-2(CH)), 8.07 (d, Ar-2(CH)).

Example 51

Preparation of compound 144:

15 To 100 mg (0.34 mM) of compound 136 in 4 mL of EtOH was added 140 mg of methoxylamine hydrochloride and 0.41 mL (5.07 mM) of pyridine. The solution was refluxed for 6 h. 1N HCl was added at RT and the organics extracted with EtOAc, washed with water, brine, and dried over MgSO_4 . The methyloxime compound was purified by crystallization from EtOAc-hexanes (1:5) to give 75 mg (0.25 mmol) of methyl oxime 144. $^1\text{H-NMR}$ (CDCl_3) δ 1.36 (s, 3(CH_3)), 2.18 (s, CH_3), 4.00 (s, - OCH_3), 7.06 (d, Ar-2(CH)), 7.20 (d, Ar-2(CH)), 7.33 (s, Ar- CH), 7.57 (d, Ar-2(CH)), 8.07 (d, Ar-2(CH)).

25 **Evaluation of Retinoid Receptor Subtype Selectivity**

Representative synthetic retinoid compounds of the current invention were analyzed and found to exhibit subtype

selectivity for retinoid receptors, and to be capable of modulating processes selectively mediated by retinoid X receptors, as discussed more fully below.

As employed herein, the phrase "processes selectively mediated by retinoid X receptors" refers to biological, physiological, endocrinological, and other bodily processes which are mediated by receptors or receptor combinations which are responsive to retinoid X receptor selective processes, e.g., compounds which selectively activate one and/or multiple members of the RXR subfamily. Modulation includes activation or enhancement of such processes as well as inhibition or repression, and can be accomplished *in vitro* or *in vivo*. *In vivo* modulation can be carried out in a wide range of subjects, such as, for example, humans, rodents, sheep, pigs, cows, and the like. It is well accepted that modulation of such processes has direct relevance to use in treating disease states.

The receptors which are responsive to retinoid X receptor selective ligands include: retinoid X receptor-alpha, retinoid X receptor-beta, retinoid X receptor-gamma, and splicing variants encoded by the genes for such receptors, as well as various combinations thereof (i.e., homodimers, homotrimers, heterodimers, heterotrimers, and the like). Also included are combinations of retinoid X receptors with other members of the steroid/thyroid superfamily of receptors with which the retinoid X receptors may interact by forming heterodimers, heterotrimers, and the higher heteromultimers. For example, the retinoic acid receptor-alpha, -beta, or -gamma isoforms form a heterodimer with any of the retinoid X receptor isoforms, (i.e., alpha, beta, or gamma, including any combination of the different receptor isoforms), and the various retinoid X receptors form a heterodimer with thyroid

receptor and form a heterodimer with vitamin D receptor. Members of the retinoid X receptor subfamily form a heterodimer with certain "orphan receptors" including PPAR (Issemann and Green, *Nature*, 347:645-49 (1990)); HNF4 (Sladek et al., *Genes & Development* 4:2353-65 (1990)); the COUP family of receptors (e.g., Miyajima et al., *Nucleic Acids Research* 16:11057-74 (1988), and Wang et al., *Nature*, 340:163-66 (1989)); COUP-like receptors and COUP homologs, such as those described by Mlodzik et al. (*Cell*, 60:211-24 (1990)) and Ladias et al. (*Science*, 251:561-65 (1991)); the ultraspiracle receptor (e.g., Oro et al., *Nature*, 347:298-301 (1990)); and the like.

As employed herein, the phrase "members of the steroid/thyroid superfamily of receptors" (also known as "nuclear receptors" or "intracellular receptors") refers to hormone binding proteins that operate as ligand-dependent transcription factors.

Furthermore, this classification includes identified members of the steroid/thyroid superfamily of receptors for which specific ligands have not yet been identified (referred to hereinafter as "orphan receptors"). All members of the intracellular receptor superfamily have the intrinsic ability to bind to specific DNA sequences.

Following binding, the transcriptional activity of a target gene (i.e., a gene associated with the specific DNA sequence) is modulated as a function of the ligand bound to the receptor. Also, see Heyman et al., *Cell*, 68:397-406 (1992), and copending U.S. Serial No. 809,980, filed December 18, 1991, whose entire disclosures are incorporated herein by reference.

The modulation of gene expression by the ligand retinoic acid and its receptors can be examined in a reconstituted system in cell culture. Such a system was used to evaluate the synthetic retinoid compounds of this invention for their interaction with the retinoid receptor subtypes RAR α , RAR β , RAR γ , RXR α , RXR β , and RXR γ .

The system for reconstituting ligand-dependent transcriptional control, which was developed by Evans *et al.*, *Science*, 240:889-95 (1988), has been termed a "co-transfection" or "cis-trans" assay. This assay is described in further detail in U.S. Patent Nos. 4,981,784 and 5,071,773, which are incorporated herein by reference. Also see Heyman *et al.*, *Cell*, 68:397-406 (1992). The co-transfection assay provides a mechanism to evaluate the ability of a compound to modulate the transcription response initiated by an intracellular receptor. The co-transfection assay is a functional, rapid assay that monitors hormone or ligand activity, is a good predictor of an *in vivo* system, and can be used to quantitate the pharmacological potency and utility of such ligands in treating disease states. Berger *et al.*, *J. Steroid Biochem. Molec. Biol.*, 41:733-38 (1992).

Briefly, the co-transfection assay involves the introduction of two plasmids by transient transfection into a retinoid receptor-negative mammalian cell background. The first plasmid contains a retinoid receptor cDNA and directs constitutive expression of the encoded receptor. The second plasmid contains a cDNA that encodes for a readily quantifiable protein, e.g., firefly luciferase or chloramphenicol acetyl transferase (CAT), under control of a promoter containing a retinoid acid response element, which confers retinoid dependence on the transcription of the reporter. In this co-transfection assay, all retinoid receptors respond to all-*trans*-retinoic acid in a similar fashion. This assay can be used to accurately measure efficacy and potency of retinoic acid and synthetic retinoids as ligands that interact with the individual retinoid receptor subtypes.

Accordingly, synthetic retinoid compounds of the current invention were evaluated for their interaction with retinoid

receptor subtypes using the co-transfection assay in which CV-1 cells were co-transfected with one of the retinoid receptor subtypes, a reporter construct, and an internal control to allow normalization of the response for transfection efficiency. The 5 following example is illustrative.

Example 52

Retinoids: All-*trans*-retinoic acid (RA) and 13-*cis*-retinoic acid (13-*cis*-RA) were obtained from Sigma. 9-*cis*-retinoic acid (9-*cis*-RA) was synthesized as described in Heyman *et al.*, *Cell*, 68:397-406 (1992).

10 Retinoid purity was established as greater than 99% by reverse phase high-performance liquid chromatography. Retinoids were dissolved in dimethylsulfoxide for use in the transcriptional activation assays.

Plasmids: The receptor expression vectors used in the co-transfection assay have been described previously (pRShRAR- α : Giguere *et al.* (1987); pRShRAR- β and pRShRAR- γ : Ishikawa *et al.* (1990); pRShRXR- α : Mangelsdorf *et al.*, (1990); pRSmRXR- β and pRSmRXR- γ : Mangelsdorf *et al.*, *Genes & Devel.*, 6:329-44 (1992)). A basal reporter plasmid Δ -MTV-LUC (Hollenberg and Evans, *Cell*, 55:899-906 (1988)) containing two copies of the TRE-palindromic response element 5'-TCAGGGTCATGACCTGA-3' (Umesono *et al.*, *Nature*, 336:262-65 (1988)) was used in transfections for the RARs, and CRBPIIFKLUC, which contains an RXRE (retinoid X receptor response element (Mangelsdorf *et al.*, *Cell*, 66:555-61 (1991)), was used in transfections for the RXRs.

25 **Co-transfection Assay In CV-1 Cells:** A monkey kidney cell line, CV-1, was used in the *cis-trans* assay. Cells were transfected with two plasmids. The trans-vector allowed efficient production of the retinoid receptor in these cells, which do not normally express this receptor protein. The *cis*-vector contains an easily

assayable gene product, in this case the firefly luciferase, coupled to a retinoid-responsive promoter, i.e., an RARE or RXRE. Addition of retinoic acid or an appropriate synthetic retinoid results in the formation of a retinoid-RAR or -RXR complex that 5 activates the expression of luciferase gene, causing light to be emitted from cell extracts. The level of luciferase activity is directly proportional to the effectiveness of the retinoid-receptor complex in activating gene expression. This sensitive and reproducible co-transfection approach permits the identification of 10 retinoids that interact with the different receptor isoforms.

Cells were cultured in DMEM supplemented with 10% charcoal resin-stripped fetal bovine serum, and experiments were conducted in 96-well plates. The plasmids were transiently transfected by the calcium phosphate method (Umesono and Evans, *Cell*, 15 57:1139-46 (1989) and Berger *et al.*, *J. Steroid Biochem. Molec. Biol.*, 41:733-38 (1992)) by using 10 ng of a pRS (Rous sarcoma virus promoter) receptor-expression plasmid vector, 50 ng of the reporter luciferase (LUC) plasmid, 50 ng of pRS β -GAL(β -galactosidase) as an internal control, and 90 ng of carrier plasmid, pGEM. Cells were 20 transfected for 6 h and then washed to remove the precipitate. The cells were then incubated for 36 h with or without retinoid. After the transfection, all subsequent steps were performed on a Beckman Biomek Automated Workstation. Cell extracts were prepared, then 25 assayed for luciferase and β -galactosidase activities, as described by Berger *et al.* (1992). All determinations were performed in triplicate in two independent experiments and were normalized for transfection efficiency by using β -galactosidase as the internal control. Retinoid activity was normalized relative to that of all-*trans*-retinoic acid and is expressed as potency (EC50), which is the 30 concentration of retinoid required to produce 50% of the maximal

observed response, and efficacy (%), which is the maximal response observed relative to that of all-*trans*-retinoic acid at 10^{-5} M. The data obtained is the average of at least four independent experiments. Efficacy values less than 5% are not statistically different than the 0% background. Compounds with an efficacy of less than 20% at concentrations of 10^{-5} M are considered to be inactive. At higher concentrations of compound, such as 10^{-4} M, these compounds are generally toxic to cells and thus the maximal efficacy at 10^{-5} M is reported in the tables and figures contained herein.

The synthetic retinoid compound 3-methyl-TTNCB, as described above, was evaluated for its ability to regulate gene expression mediated by retinoid receptors. As shown in Figure 1, this compound is capable of activating members of the RXR subfamily, i.e., RXR α , RXR β , and RXR γ , but clearly has no significant activity for members of the RAR subfamily, i.e., RAR α , RAR β , and RAR γ . Assays using all-*trans*-retinoic acid (Figure 2) and 9-*cis*-retinoic acid (Figure 3) were run for reference, and demonstrate that these retinoic acid isomers activate members of both the RAR and RXR subfamilies.

Potency and efficacy were calculated for the 3-methyl-TTNCB compound, as summarized in the following table. For reference, the data for 9-*cis*-retinoic acid are also included.

TABLE 1

Potency (nM) Efficacy3-Methyl-TTNCB

5	RXR α	330	130%
	RXR β	200	52%
	RXR γ	260	82%
	RAR α	>10,000	<2%
	RAR β	>10,000	<4%
	RAR γ	>10,000	<4%
<u>10</u>			
<u>9-cis-retinoic acid</u>			
15	RXR α	150	140%
	RXR β	100	140%
	RXR γ	110	140%
20	RAR α	160	100%
	RAR β	5	82%
	RAR γ	47	120%

As shown by the data in Table 1, 3-methyl-TTNCB readily and at low concentrations activates RXRs. Further, 3-methyl-TTNCB is more potent an activator of RXRs than RARs, and preferentially activates RXRs in comparison to RARs, in that much higher concentrations of the compound are required to activate the RARs. In contrast, 9-cis-retinoic acid does not preferentially activate the RXRs, as also shown in Table 1. Rather, 9-cis-retinoic acid activates the RAR β and RAR γ isoforms at lower concentrations and more readily than the RXR β and RXR γ isoforms, and has substantially the same, within the accuracy of the measurement, activity for the RAR α isoform in comparison to the RXR α isoform.

An extract reported to contain 9-cis-retinoic acid has previously been reported as at least 10-fold more potent in inducing RXR α than RAR α (Heyman *et al.*, *Cell*, 68:397,399 (January 24, 1992)). Presently available data indicate that 9-cis-retinoic acid does not preferentially activate RXRs in comparison to RARs, as

shown and discussed above. The compounds of this invention preferentially activate RXRs in comparison to RARs, and are preferably at least three times more potent as activators of RXRs than RARs, and more preferably at least five times more potent as activators of RXRs than RARs.

5 Potency and efficacy have also been calculated for the 3-methyl-TTNEB, 3-bromo-TTNEB, 3-methyl-TTNCHBP, 3-methyl-TTNEHBP, TPNEP, and TPNEP compounds, as summarized below in Table 2.

TABLE 2

Potency (nM) Efficacy3-Methyl-TTNEB

5	RXR α	40	83%
	RXR β	21	102%
	RXR γ	34	80%
10	RAR α	>10,000	6%
	RAR β	>10,000	17%
	RAR γ	>10,000	19%

3-Bromo-TTNEB

15	RXR α	64	88%
	RXR β	54	49%
	RXR γ	52	71%
20	RAR α	>10,000	3%
	RAR β	>10,000	18%
	RAR γ	>10,000	15%

3-Methyl-TTNCHBP

20	RXR α	1100	113%
	RXR β	1100	155%
	RXR γ	300	128%
25	RAR α	>10,000	<2%
	RAR β	>10,000	7%
	RAR γ	>10,000	17%

3-Methyl-TTNEHBP (63)

25	RXR α	140	125%
	RXR β	71	121%
	RXR γ	48	163%
30	RAR α	>10,000	<2%
	RAR β	1,900	25%
	RAR γ	>10,000	10%

TPNEP (58)

35	RXR α	5	75%
	RXR β	5	138%
	RXR γ	6	100%
35	RAR α	>10,000	<2%
	RAR β	>10,000	<2%
	RAR γ	1,500	24%

TPNCP (62)

5	RXR α	4	63%
	RXR β	4	93%
	RXR γ	3	49%
	RAR α	>10,000	<2%
	RAR β	>10,000	<2%
	RAR γ	>10,000	<2%

As shown by the data in Table 2, 3-methyl-TTNEB, 3-bromo-TTNEB, 3-methyl-TTNCHBP, 3-methyl-TTNEHBP, TPNEP, and TPNCP each readily and preferentially activate the RXRs, and are more potent as activators of RXRs than of RARs. The diminished activity of these compounds for the RARs in comparison to the RXRs is also shown for some of these compounds in Figures 4-7.

The potency and efficacy of derivative compounds 133 and 15 137 have also been calculated, as summarized below in Table 3.

TABLE 3
Potency (nM) Efficacy

Compound 133

20	RXR α	440	86%
	RXR β	470	56%
	RXR γ	290	91%
	RAR α	>10,000	<2%
	RAR β	>10,000	<2%
	RAR γ	>10,000	<2%

Compound 137

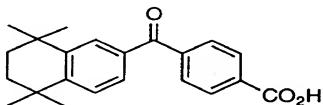
25	RXR α	270	47%
	RXR β	270	57%
	RXR γ	190	50%
	RAR α	>10,000	<2%
	RAR β	>10,000	<2%
30	RAR γ	>10,000	<2%

As shown, these compounds preferentially activate RXRs in comparison to RARs.

The selective activity of the compounds of this invention for Retinoid X Receptors is not exhibited by other known compounds.

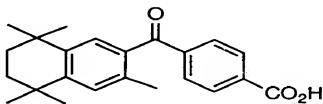
For example, compounds such as those described in U.S. Patent No. 4,833,240 (Maignan et al.) appear structurally similar to the compounds of this invention, but lack a functional group (such as methyl, ethyl, isopropyl, bromo, chloro, etc.) at the 3-position. 5 Such compounds have little or no potency and lack any selectivity for RXRs.

For example, a representative compound of U.S. Patent No. 4,833,240 (Maignan) is shown below, along with the compound 3-methyl-TTNCB of this invention.



10

Maignon Ex. II



3-methyl-TTNCB

The potency and efficacy of the Maignon compound and that of 3-methyl-TTNCB are summarized below:

		<u>Potency (nM)</u>	<u>Efficacy</u>
<u>Maignon Ex. II</u>			
	RXR α	3,000	82%
	RXR β	3,000	44%
	RXR γ	3,000	64%
5	RAR α	>10,000	11%
	RAR β	1,900	58%
	RAR γ	2,000	56%
<u>3-Methyl-TTNCB</u>			
10	RXR α	330	130%
	RXR β	200	52%
	RXR γ	260	82%
	RAR α	>10,000	<2%
	RAR β	>10,000	<4%
	RAR γ	>10,000	<4%

15 As shown, the Maignon compound is virtually inactive and shows no selectivity for RXRs. In contrast, the compounds of this invention such as 3-methyl-TTNCB, which have a substituent at the 3-position, are potent activators of RXRs and exhibit the unexpected RXR selectivity shown in Table 1 (as well as Tables 2 and 3) and discussed above.

20 It can be expected that synthetic retinoid ligands, such as those exemplified in Tables 1, 2, and 3 which preferentially affect some but not all of the retinoic acid receptor isoforms, can, in pharmacological preparations, provide pharmaceuticals with 25 higher therapeutic indices and a better side effect profile than currently used retinoids. For example, the compounds of the present invention have been observed to be less irritating to the skin than previously known retinoids.

30 The retinoid compounds of this invention are useful for the treatment of certain dermatological conditions such as keratinization disorders, i.e., differentiation/proliferation. A standard assay to determine the activity of these compounds is the measurement of the enzymatic activity for transglutaminase; this is a measure of the antiproliferative action

of retinoids. Retinoids have been shown to inhibit the pathway of differentiation, which is indicated by a decrease in several biochemical markers that are associated with the expression of squamous cell phenotype, such as transglutaminase. (Yuspa et al., *Cancer Research*, 43:5707-12 (1983)). As can be seen from Figure 8, the 3-methyl-TTNCB compound is capable of inhibiting transglutaminase activity and inhibits 50% of the enzyme activity at 1×10^{-7} M.

The retinoid compounds of this invention have been demonstrated in *in vitro* tests to block (or antagonize) AP-1 activity, a set of oncogenes which drive cellular proliferation. Many proliferative disorders are the results of oncogenes/oncogene activation, and therefore a compound which blocks the AP-1 oncogene pathway can be used to treat diseases associated with proliferative disorders including cancers, inflammatory diseases, psoriasis, etc. For example, the compound 3-methyl-TTNEB was evaluated using the co-transfection assay in which HeLa cells were co-transfected with a plasmid expressing RXR α under the control of a constitutive promoter, and a plasmid which expresses the reporter enzyme luciferase under the control of a conditional promoter (collagenase) containing an AP-1 responsive element. E.g., Angel et al., *Mol. Cell. Biol.*, 7:2256 (1987); Lafyatis et al., *Mol. Endocrinol.*, 4:973 (1990). Following AP-1 activation, the assay results showed antagonism of AP-1 activity by the 3-methyl-TTNEB compound via RXR α in a dose-dependent manner. Other compounds of this invention have shown similar antagonism of AP-1 activity. These results demonstrate that RXR-selective compounds such as 3-methyl-TTNEB can be used as anti-proliferatives to limit cell growth and treat diseases associated with hyperproliferation.

The compounds of this invention also exhibit good comedolytic activity in the test on Rhino mice described by Kligman

et al. (J. of Inves. Derm., 73:354-58 (1979)) and Mezick et al. (J. of Inves. Derm., 83:110-13 (1984)). The test on Rhino mice has been a model for screening comedolytic agents. The activity of the 3-methyl-TTNCB retinoid compound, as well as 9-cis and all-trans retinoic acid is 5 shown in Figure 9. A 0.1% solution of 3-methyl-TTNCB is capable of inhibiting the utriculi diameter by approximately 50%. It has also been observed that 3-methyl-TTNCB is less irritating to the skin of Rhino mice than 9-cis- or all-trans-retinoic acid.

The co-transfection assay allows examination of the 10 ability of a compound to modulate gene expression in a retinoid receptor dependent fashion. To examine the ability of the compounds of this invention to directly interact with the receptors, we have examined the ligand binding properties of all six retinoid receptors. The receptors were expressed employing a 15 baculovirus expression system in which we have demonstrated that RxR α binds 9-cis-retinoic acid with high affinity (Heyman et al., Cell, 68:397 (1992)). The binding parameters of receptors expressed in baculovirus systems and mammalian systems are essentially identical.

20 The synthetic retinoids of the current invention have also been tested using radioligand displacement assays. By testing the abilities of various synthetic retinoids to compete with the radiolabeled retinoic acid for binding to various receptor isoforms, the ability of the compounds to directly interact with 25 the receptor can be examined, and the relative dissociation constant for the receptor itself can be determined. This is an important supplementary analysis to the co-transfection assay since it can detect different properties/determinants of retinoid activity than are measured in the co-transfection assay. These 30 determinants/differences in the two assay systems may include (1)

activating or inactivating metabolic alterations of the test compounds, (2) binding to serum proteins which could alter the free concentration or other properties of the test compound, (3) differences in cell permeation among test compounds, (4) intrinsic 5 differences in the affinity of the test compounds for the receptor proteins, i.e., in K_d , can be directly measured, and (5) conformational changes produced in the receptor after binding of the test compound, reflected in the effects on reporter gene expression; (i.e., a functional measurement of receptor 10 activation).

The 3-methyl-TTNCB compound is capable of displacing ^3H -9-cis-retinoic acid bound to the RXRs, but is not capable of displacing radiolabeled ligand that is bound to the RARs. This indicates that the 3-methyl-TTNCB compound preferentially binds 15 RXRs in comparison to RARs, a property which would be expected of a ligand selective for the RXRs. The K_d values were determined by application of the Cleng-Prusoff equation. These values were based on a determination of the IC_{50} value as determined graphically from a log-logit plot of the data.

20 Binding data were obtained for various compounds using the method discussed in Wecksler & Norman, *Anal. Biochem.* 92:314-23 (1979). Results are shown below in Table 4.

TABLE 4

<u>3-Methyl-TTNCB</u>		<u>Binding (Kd₅₀ nM)</u>
5	RXR α	350
	RXR β	230
	RXR γ	365
	RAR α	>10,000
	RAR β	>10,000
	RAR γ	>10,000
<u>3-Methyl-TTNEB</u>		
10	RXR α	41
	RXR β	20
	RXR γ	22
15	RAR α	5,500
	RAR β	5,400
	RAR γ	3,200
<u>TPNEP (58)</u>		
20	RXR α	22
	RXR β	21
	RXR γ	39
25	RAR α	7,800
	RAR β	4,900
	RAR γ	6,000
<u>TPNCP (62)</u>		
30	RXR α	3
	RXR β	3
	RXR γ	3
35	RAR α	>10,000
	RAR β	>10,000
	RAR γ	>10,000

The compounds in Table 4 were shown to readily and preferentially activate RXRs, and to be more potent as activators of RXRs than of RARs using the co-transfection assay, as discussed above. The binding results in Table 4 show these compounds to also preferentially bind RXRs versus RARs. Taken together the ligand binding properties of these compounds and their ability to selectively modulate members of the RXR subfamily demonstrate the identification of a class of compounds with the unique biological properties. The binding properties and especially the

transcriptional activation assays are a good predictor of the pharmacological activity of a compound (Berger et al. (1992)).

It has been recognized that the co-transfection assay provides a functional assessment of the ligand being tested as either an agonist or antagonist of the specific genetic process sought to be affected, and is a predictor of *in vivo* pharmacology (Berger et al. (1992)). Ligands which do not significantly react with other intracellular receptors, as determined by the co-transfection assay, can be expected to result in fewer pharmacological side effects. Because the co-transfection assay is run in living cells, the evaluation of a ligand provides an early indicator of the potential toxicity of the candidate at concentrations where a therapeutic benefit would be expected.

Processes capable of being modulated by retinoid receptors, in accordance with the present invention, include *in vitro* cellular differentiation, the regulation of morphogenetic processes including limb morphogenesis, regulation of cellular retinol binding protein (CRBP), and the like. As readily recognized by those of skill in the art, the availability of ligands for the retinoid X receptor makes it possible, for the first time, to elucidate the processes controlled by members of the retinoid X receptor subfamily. In addition, it allows development of assays for the identification of antagonists for these receptors.

The processes capable of being modulated by retinoid receptors, in accordance with the present invention, further include the *in vivo* modulation of lipid metabolism; *in vivo* modulation of skin related processes (e.g., acne, psoriasis, aging, wrinkling, and the like); *in vivo* modulation of programmed cell death (apoptosis); *in vivo* modulation of malignant cell development, such as occurs, for example, in acute promyelocytic leukemia, mammary cancer, prostate

cancer, lung cancer, cancers of the aerodigestive pathway, skin cancer, bladder cancer, and sarcomas; *in vivo* modulation of premalignant lesions, such as occurs with oral leukoplakia and the like; *in vivo* modulation of auto-immune diseases such as rheumatoid 5 arthritis; *in vivo* modulation of fatty acid metabolism; and the like. Such applications can be expected to allow the modulation of various biological processes with reduced occurrence of undesirable side effects such as teratogenic effects, skin irritation, mucosal dryness, lipid disturbances, and the like. *In vivo* applications can 10 be employed with a wide range of subjects, such as, for example, humans, rodents, sheep, pigs, cows, and the like.

For example, regarding the *in vivo* modulation of lipid metabolism referred to above, apolipoprotein A-1 ("apo A-1") is a major protein component of plasma high density lipoprotein (HDL) 15 cholesterol. The circulating level of HDL in humans has been shown to be inversely correlated to the risk of atherosclerotic cardiovascular disease (ASCVD), the leading cause of morbidity and mortality in the United States, with a 3-4% increase in ASCVD for every 1% decrease in HDL cholesterol. Gordon *et al.*, *New Engl. J. Med.*, 20 321:1311 (1989). While there are currently no good therapeutic regimens that increase HDL cholesterol, it can be expected that regulating synthesis of apoAI can be utilized to affect plasma concentrations of HDL cholesterol and to decrease the risk of ASCVD. Rubin *et al.*, *Nature*, 353:265 (1991).

25 It has been established that regulation of transcription of apoAI is controlled by members of the intracellular receptor superfamily, and further that the apoAI gene transcription start site "A" is a highly selective retinoic acid-responsive element that responds to retinoid X receptors. Rottman *et al.*, *Mol. Cell. Biol.*,

11:3814-20 (1991). Because RXRs can form heterodimers with transrepressors such as ARP-1 and COUP-TF and transactivators such as HNF-4, and an RXR response element resides in the apoA1 promoter, retinoids or ligands which selectively activate members 5 of the RXR family of retinoic acid receptors may regulate apoA1 transcription. We have demonstrated in *in vivo* studies that ligands of this invention which have selective activity for RXRs can be used to modulate apoA1/HDL cholesterol and to significantly raise plasma HDL levels, as demonstrated in the following example.

Example 53

Male Sprague-Dawley rats (160-200 gram) were obtained from Harlan. Animals were fed standard laboratory diets (Harlan/Teklad) and kept in an environmentally controlled animal house with a light period lasting from 6 a.m. to 6 p.m. Animals were treated with drugs prepared as suspensions in olive oil.

To verify that RXR activation can increase plasma apoA1/HDL cholesterol, an initial study was carried out that included dosing rats for 4 days with an RAR-selective compound, all-*trans* retinoic acid, the non-selective RAR/RXR agonist, 9-*cis*-retinoic acid, and either of two RXR-selective agents, 3-methyl-TTNCB or 3-methyl-TTNEB. Each drug was administered at a dose of 100 mg/kg, i.p. Positive control groups received olive oil as a vehicle. Twenty-four hours after the last treatment, rats were sacrificed by CO₂ inhalation, blood was collected from the inferior vena cava into a tube containing 0.1 ml of 0.15% EDTA and centrifuged at 1500 x g for 20 min. at 4°C. Plasma was separated and stored at 4°C for evaluation of plasma total cholesterol and high density lipoprotein cholesterol (HDL-cholesterol).

Plasma total cholesterol was measured enzymatically utilizing Boeringer Mannheim Diagnostics High Performance Cholesterol Methods with an ABBOTT VP Bichromatic Analyzer. HDL cholesterol was measured after preparation of the HDL-containing fraction by heparin-manganese precipitation of plasma. HDL-cholesterol in this fraction was estimated as mentioned earlier. All HDL separations were checked for contamination by other lipoproteins with agarose gel electrophoresis.

The results of this study are shown in Figure 10. As shown, rats receiving the RXR-selective compounds exhibited

substantial and statistically significant increases in HDL levels, particularly when receiving 3-methyl-TTNEB.

Because the RXR-selective ligand 3-methyl-TTNEB was the most efficacious, additional 4 day experiments were conducted with this agent at doses of 0.3, 1, 3, 6, 10, 30, 100, or 300 mg/kg i.p. in 1.0 ml olive oil or 1, 3, 10, 30, 100, 300 mg/kg p.o. in 1.0 ml olive oil for 4 days. An additional 30 day p.o. study was conducted with 10, 30, or 100 mg/kg 3-methyl-TTNEB to determine whether tolerance would develop to its pharmacological actions. For the rats receiving 3-methyl-TTNEB in various doses for four days, it was also observed that 3-methyl-TTNEB increased plasma concentrations of HDL-cholesterol in a dose-dependent manner with significant increases being made at the lowest dose, 0.3 mg/kg i.p. At its optimally efficacious dose, 3-methyl-TTNEB increased plasma HDL-cholesterol concentrations from 58 mg/dl to 95 mg/dl - an increase of greater than 60%. Measurement of total cholesterol showed an increase due to the increase of the HDL-cholesterol fraction. Measurement of triglycerides showed either no change or a slight decrease. The 30-day study with 3-methyl-TTNEB did not indicate development of tolerance to its pharmacological action. This study demonstrated that 3-methyl-TTNEB increased plasma concentrations of HDL-cholesterol in a dose-dependent manner following either i.p. or p.o. administration.

The effect on circulating apoA1 of orally administered 3-methyl-TTNEB was also studied. Male Sprague-Dawley rats were treated daily with 3 mg/kg body weight of 3-methyl-TTNEB for four days. Serum samples were taken and analyzed by Western Blot using antisera specific to rat apoA1. The treatment with 3-methyl-TTNEB resulted in a significant increase in circulating apoA1 levels.

These studies demonstrate that treatment with an RXR specific compound such as 3-methyl-TTNEB increases plasma

concentrations of apoA1/HDL cholesterol. Since such animal studies are an accepted predictor of human response, it would therefore be expected that such compounds could be used to therapeutically increase HDL-cholesterol in patients who either have, or are at 5 risk for, atherosclerosis.

Additional *in vitro* studies were also performed utilizing the co-transfection assay previously described within this application to demonstrate the effect of RXR-selective ligands on regulation of transcription of apoA1, as described in the following example.

10 **Example 54:**

This work focused on studying the transcriptional properties of the retinoid receptors RAR and RXR on a reporter molecule (e.g., luciferase) under control of a basal promoter containing the RXR response element from the apoA1 gene ("A" site).

15 Plasmid constructs coding for the various receptors were transfected into a human hepatocyte cell line (HepG-2) along with the reporter plasmid. Reporter plasmids contained multimers of the apoA1 "A" site (-214 to -192 relative to transcription start site) shown to bind RXR. Widom *et al.*, *Mol. Cell. Biol.* 12:3380-89 (1992); Ladhas 20 & Karathanasis, *Science* 251:561-65 (1991). After transfection, treatment, harvest, and assay, the data obtained was normalized to transfected beta-galactosidase activity so as to control for transfection efficiency. The results demonstrated activation in the system with the RXR-specific ligands 3-methyl-TTNCB and 3- 25 methyl-TTNEB in a concentration-dependent fashion, demonstrating that the RXR specific ligands could regulate the transcriptional properties via the "A" site from the apoA1 gene. These compounds had no effect when RAR was used in the transfection, demonstrating

receptor specificity. The transcriptional regulation by RXR was dependent on the presence of the hormone response element.

These *in vivo* and *in vitro* studies demonstrate that the RxR-selective compounds of this invention can be used to elevate apoA1/HDL cholesterol and in the therapeutic treatment of related cardiovascular disorders.

Regarding the modulation of programmed cell death (apoptosis), the retinoid compounds of this invention have been demonstrated to induce apoptosis in particular cell types including leukemic cells and squamous epithelial carcinomas. Normally in cells there is a fine balance between cellular processes of proliferation, differentiation, and cell death, and compounds which affect this balance may be used to treat certain cancers.

Specifically, the ability of 3-methyl-TTNEB to induce differentiation, inhibit proliferation, and induce apoptosis in an acute promyelocytic leukemia cell line, HL60, was studied.

Cellular proliferation was measured by a thymidine incorporation assay (Shrivastav et al., *Cancer Res.*, 40:4438 (1980)), and 3-methyl-TTNEB was found to have no effect on cellular proliferation.

This contrasts all-*trans*-retinoic acid, which inhibits thymidine incorporation. Cellular differentiation was measured by the ability of the cells to reduce nitroblue tetrazolium (NBT) (Breitman et al., *Proc. Natl. Acad. Sci.*, 77:2936 (1980)), and 3-methyl-TTNEB was found not to induce undue differentiation. The EC₅₀ for 3-methyl-TTNEB mediated differentiation was >1000 μ M, compared to 2.0 μ M for all-*trans*-retinoic acid. However, 3-methyl-TTNEB was found to induce transglutaminase activity in the HL60 cells (Murtaugh et al., *J. Biol. Chem.* 258:11074 (1983)) in a concentration-dependent manner, which correlates with the induction of apoptosis or programmed cell death. It was further found that 3-methyl-TTNEB

was able to induce apoptosis as measured by DNA fragmentation and morphological changes. Other retinoid compounds of this invention showed similar results, and similar results were also shown in other cell lines such as squamous epithelial cell lines and ME180

5 cells, a human cervical carcinoma.

These results show that RXR specific compounds such as 3-methyl-TTNEB induce apoptosis with a minimal direct effect on inhibition of proliferation and differentiation induction. Compounds which are capable of inducing apoptosis have been shown

10 to be effective in cancer chemotherapy (*e.g.*, anti-hormonal therapy for breast and prostate cancer).

In contrast, in another cell type retinoids have been shown to inhibit activation driven T-cell apoptosis and 9-cis-retinoic acid was approximately ten fold more potent than all-

15 *trans*-retinoic acids (Ashwell *et al.*, *Proceedings National Academy of Science*, Vol. 90, p. 6170-6174 (1993)). These data imply that RXRs are involved in this event. Thus retinoids could be used to block and/or immunomodulate T-cell apoptosis associated with certain disease states (*e.g.*, AIDS).

It has been surprisingly found that the administration of a ligand which has specific activity for RXRs but essentially no activity for RARs, in combination with a ligand that has specific activity for RARs but not RXRs, provides a cellular response at extremely low dosages, dosages at which the ligands individually

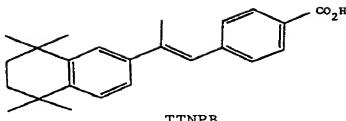
20 provide no significant response. Specifically, the concentration-related effect of an RXR-specific ligand and a RAR-specific ligand on proliferation of a myeloma cell line (RPMI 8226) was studied in *in vitro* studies using a thymidine incorporation assay. This assay examines the incorporation of radiolabeled thymidine into DNA, and

25 30 by determining the ability of a compound to inhibit thymidine

incorporation into DNA, provides a measure of cell proliferation. (L.M. Bradley, Selected Methods in Cellular Immunology, Ch. 10.1, pp. 235-38, Mishell & Shiigi (eds.), Freeman & Co., New York, 1980). Compounds which inhibit cell proliferation have well-known 5 utility in the treatment of certain cancers.

As shown previously (Table 2), 3-methyl-TTNEB activates members of the RXR subfamily and has no significant activity for members of the RAR subfamily. Examination of the effects of 3-methyl-TTNEB on the proliferation of myeloma cells show a 10 concentration dependent inhibition of thymidine incorporation. The IC₅₀ (the concentration of 3-methyl-TTNEB required to produce 50% inhibition of the maximal response) is 10⁻⁷ M, as shown in Figure 11. Concentrations less than 10⁻⁸ M provide essentially no effect on cell proliferation, as also shown in Figure 11.

15 It is well known that the compound TTNPB activates members of the RAR subfamily and has no significant activity for members of the RXR subfamily. The compound TTNPB is shown below, and its activity is shown in Table 5.

TABLE 5

	<u>Potency (nM)</u>	<u>Efficacy</u>
TTNPB		
5	RXR α RXR β RXR γ	>10,000 >10,000 >10,000
	RAR α RAR β RAR γ	52 4 0.4
		30 40 50

10 The effect of TTNPB on cell proliferation is shown in Figure 11. The IC₅₀ value of TTNPB is about 5×10^{-11} M, and a concentration of less than 10^{-11} M produces essentially no effect on cell proliferation.

15 However, it has been found that when 3-methyl-TTNEB and TTNPB are present together, each at a concentration where the compound alone produces substantially no anti-proliferative effect, the combination of the two compounds effectively blocks cell proliferation. The combination of the two compounds appears to produce a greater than additive, or synergistic, effect.

20 For example, as shown in Figure 12, the presence of TTNPB at a concentration of 10^{-11} M produces a 9% inhibition on thymidine incorporation. However, combining it with 3-methyl-TTNEB at a concentration of 10^{-8} M (which results in no effect on cell proliferation) produces a greatly enhanced inhibitory effect of 49%. Likewise, it has also been found that the inhibitory effect of 3-methyl-TTNEB is greatly increased by the presence of TTNPB at a concentration which alone produces no effect.

Since it is well-known that toxic side effects of compounds such as TTNPB are concentration-dependent, the synergistic effect resulting from combining such RAR-specific compounds with RXR-specific compounds can be expected to permit 5 lower dosages that are efficacious and to therefore reduce toxic side effects. For example, in cancer chemotherapy, use of two such compounds, in combination, at relatively low doses can be expected to produce the desired beneficial effect, while minimizing undesired side effects which result at higher doses of the 10 compounds.

In vitro studies utilizing the co-transfection assay have also shown this same synergistic effect. For example, utilizing the co-transfection assay described previously and employing RAR- α and RXR- α and a reporter consisting of the ApoA1 response element 15 "A" site in the context of TKLUC (Ladias & Karathanasis, *Science* 251:561-65 (1991), transfections were performed in HEPG2 cells. In this study, 100 ng of the designated receptor were used and RSVCAT was used as a carrier to keep the amount of RSV promoter constant. All compounds were added at a final concentration of 10^{-7} M. The 20 RXR specific compound 3-methyl-TTNEB (Table 2, above) and the RAR specific compound TTNPB (Table 5, above) were utilized. As shown below in Table 6, the relative normalized response observed utilizing the co-transfection assay also demonstrated a synergistic effect when a combination of the two compounds was utilized, 25 compared to the response achieved utilizing the compounds individually.

TABLE 6

<u>Compound</u>	<u>Reporter Activity (Fold Induction)</u>
3-methyl-TTNEB	5
TTNPB	32
3-methyl-TTNEB + TTNPB	75

As will be discernable to those skilled in the art from the foregoing discussions, the biological response of an RAR selective compound at a given concentration can be synergistically enhanced by combining the compound with an RXR selective compound. Similarly, the biological response of an RXR selective compound can be enhanced by combining the compound with an RAR selective compound. Thus, it becomes possible to achieve a desirable biological response, using a combination of RAR and RXR selective compounds, at lower concentrations than would be the case using the compounds alone. Among the advantages provided by such combinations of RAR and RXR selective compounds are desirable therapeutic effects with fewer side effects. In addition, novel effects that are not obtainable with either agent alone may be achieved by combinations of RAR and RXR selective compounds.

It has been further demonstrated that RXR-specific compounds also synergistically enhance the response of other hormonal systems. Specifically, peroxisome proliferator-activated receptor (PPAR) is a member of the intracellular receptor super family that plays a role in the modulation of lipid homeostasis. PPAR has been shown to be activated by amphipathic carboxylates, such as clofibrate acid, and gemfibrozil. These agents, called peroxisome proliferators, have been used in man as hypolipidemic agents. The addition of 9-cis-retinoic acid (a retinoid ligand which activates both RAR and RXR receptors) and clofibrate acid to

HepG2 cells transfected with RXR α and PPAR expression plasmids, results in the activation of receptor gene which was greater than the sum of the activation with each ligand separately. (Kliewer et al., *Nature* 358:771 (1992)). Similarly, when the above two receptors were co-transfected into HepG2 cells, the addition of both an RXR-specific ligand (3-methyl-TTNEB) and clofibrate acid was found to produce a greater than additive response as determined by activation of a target reporter gene, as shown below in Table 7.

TABLE 7

10	<u>Compound</u>	<u>Normalized Response (%)</u>
	clofibrate Acid	100
	3-methyl-TTNEB	90
	clofibrate acid + 3-methyl-TTNEB	425

15 A similar synergistic effect was observed with RXR and RXR-specific ligands and the Vitamin D receptor (VDR) and its cognate ligands. When RXR β and VD receptors were co-transfected into CV-1 cells containing a hormone response element, the addition of RXR selective 3-methyl-TTNCB and 1,25-dihydroxy-vitamin D (1,25-D) produced a greater than additive response than was observed for 20 each of the individual ligands, as shown below in Table 8.

TABLE 8

	<u>Compound</u>	<u>Normalized Response (%)</u>
	1,25-D	100
25	3-Methyl-TTNCB	13
	1,25-D + 3-methyl-TTNCB	190

As shown, the above results indicate that each pair of receptors (RXR α /PPAR and RXR β /VDR, respectively), in the presence of ligands known to specifically activate their respective receptors, are capable of producing a synergistic response. The 5 results indicate that the response of a single agent can be enhanced by the combination of the two agents, or that comparable biological or therapeutic responses can be achieved by use of lower doses of such agents in combination.

The observation that RXR-specific ligands are able to act 10 synergistically with RAR ligands, PPAR ligands, and Vitamin D ligands indicates that RXR-specific ligands have usefulness not only as single therapeutic agents but also in combination therapy to obtain enhanced biological or therapeutic response by the addition of the RXR-specific ligand. Such combination therapy also 15 may provide an added benefit of decreasing the side effects associated with the primary agent by employing lower doses of that agent. For example, use of Vitamin D or a related Vitamin D receptor ligand in conjunction with an RXR selective compound for the treatment of a variety of disorders including skin diseases 20 (acne, psoriasis), hyperproliferative disorders (benign and malignant cancers) and disorders of calcium homeostasis may decrease the adverse side effects associated with Vitamin D therapy alone.

As a further example, the RXR-specific compounds of this 25 invention have been demonstrated *in vitro* to act synergistically with compounds which affect cellular proliferation, such as Interferon. Specifically, the growth properties of two human tumor cell lines (ME180, a squamous cell carcinoma, and RPMI18226, a multiple myeloma) were monitored in the presence of the compound 3-methyl-TTNEB alone and in combination with Interferon α 2b, utilizing 30 standard cell culture procedures. The effects on growth of these

cells were monitored by evaluation of cell number, and also by evaluation of growth in semi-solid medium for the RPMI18226 cell line. Both 3-methyl-TTNEB and Interferon α 2b were found to inhibit cell growth in a concentration-dependent manner, and each alone to 5 produce a significant depression in cell proliferation. In addition, when the cells were treated with both compounds, an additive or a greater than additive effect on the depression in cell proliferation was observed. Treatment with other 10 chemotherapeutic agents including anti-proliferative agents and/or cell-cycle modulators (e.g., methotrexate, fluorouracil (5FU), ARA-C, etc.) in combination with RXR-specific compounds would be expected to produce similar results. The enhanced anti- 15 proliferative effect can be expected to permit lower therapeutic doses in treatment of proliferative disorders, such as squamous cell and other carcinomas. In addition, combination therapy could allow the use of lower doses of these compounds to achieve a comparable beneficial effect along with fewer side effects/toxic effects, thereby enhancing the therapeutic index of the therapy. 20 The therapeutic index is defined as the ratio of efficacy to toxicity of a compound.

Since RXR is known to form heterodimers with various members of the intracellular receptor super family, it can be expected that the synergistic response observed with use of RXR-selective ligands may be achieved with other receptors with which 25 heterodimers are formed. These include PPARs, RARs, Vitamin D, thyroid hormone receptors, HNF4, the COUP family of receptors, as referenced above, and other as yet unidentified members of the intracellular super family of receptors.

As will be further discernible to those skilled in the 30 art, the compounds disclosed above can be readily utilized in pharmacological applications where selective retinoid receptor

activity is desired, and where it is desired to minimize cross reactivities with other related intracellular receptors. *In vivo* applications of the invention include administration of the disclosed compounds to mammalian subjects, and in particular to

5 humans.

The compounds of the present invention are small molecules which are relatively fat soluble or lipophilic and enter the cell by passive diffusion across the plasma membrane.

Consequently, these ligands are well suited for administration

10 orally and by injection, as well as topically. Upon

administration, these ligands can selectively activate retinoid X receptors, and thereby selectively modulate processes mediated by these receptors.

The pharmaceutical compositions of this invention are prepared in conventional dosage unit forms by incorporating an active compound of the invention, or a mixture of such compounds, with a nontoxic pharmaceutical carrier according to accepted procedures in a nontoxic amount sufficient to produce the desired pharmacodynamic activity in a mammalian and in particular a human subject. Preferably, the composition contains the active ingredient in an active, but nontoxic, amount selected from about 5 mg to about 500 mg of active ingredient per dosage unit. This quantity depends on the specific biological activity desired and the condition of the patient.

The pharmaceutical carrier or vehicle employed may be, for example, a solid or liquid. A variety of pharmaceutical forms can be employed. Thus, when using a solid carrier, the preparation can be plain milled, micronized in oil, tableted, placed in a hard gelatin or enteric-coated capsule in micronized powder or pellet form, or in the form of a troche, lozenge, or suppository. When

using a liquid carrier, the preparation can be in the form of a liquid, such as an ampule, or as an aqueous or nonaqueous liquid suspension. For topical administration, the active ingredient may be formulated using bland, moisturizing bases, such as ointments or 5 creams. Examples of suitable ointment bases are petrolatum, petrolatum plus volatile silicones, lanolin, and water in oil emulsions such as Eucerin (Beiersdorf). Examples of suitable cream bases are Nivea Cream (Beiersdorf), cold cream (USP), Purpose Cream (Johnson & Johnson) hydrophilic ointment (USP), and Lubriderm 10 (Warner-Lambert).

The following examples provide illustrative pharmacological composition formulations:

Example 55

Hard gelatin capsules are prepared using the following 15 ingredients:

	<u>Quantity</u>
<u>(mg/capsule)</u>	
3-methyl-TTNCB	140
Starch, dried	100
20 Magnesium stearate	<u>10</u>
Total	250 mg

The above ingredients are mixed and filled into hard gelatin capsules in 250 mg quantities.

Example 56

25 A tablet is prepared using the ingredients below:

	<u>Quantity</u>
<u>(mg/tablet)</u>	
3-methyl-TTNCB	140
Cellulose, microcrystalline	200
30 Silicon dioxide, fumed	<u>10</u>
Stearic acid	<u>10</u>

Total 360 mg

The components are blended and compressed to form tablets each weighing 360 mg.

Example 57

Tablets, each containing 60 mg of active ingredient, are made as follows:

		Quantity (mg/tablet)
	3-methyl-TTNCB	60
10	Starch	45
	Cellulose, microcrystalline	35
	Polyvinylpyrrolidone (PVP) (as 10% solution in water)	4
	Sodium carboxymethyl starch (SCMS)	4.5
15	Magnesium stearate	0.5
	Talc	<u>1.0</u>
	Total	150 mg

The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The 20 solution of PVP is mixed with the resultant powders, which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve. The SCMS, magnesium stearate, and talc, previously passed through a No. 25 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Example 58

Suppositories, each containing 225 mg of active ingredient, may be made as follows:

3-methyl-TTNCB	225 mg
Saturated fatty acid glycerides	<u>2.000 mg</u>
Total	2,225 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then 10 poured into a suppository mold of normal 2g capacity and allowed to cool.

Example 59

An intravenous formulation may be prepared as follows:

3-methyl-TTNCB	100 mg
Isotonic saline	1,000 ml
Glycerol	100 ml

The compound is dissolved in the glycerol and then the solution is slowly diluted with isotonic saline. The solution of the above ingredients is then administered intravenously at a rate 20 of 1 ml per minute to a patient.

The compounds of this invention also have utility when labeled as ligands for use in assays to determine the presence of RXRs. They are particularly useful due to their ability to selectively bond to members of the RXR subfamily and can therefore 25 be used to determine the presence of RXR isoforms in the presence of other related receptors.

Due to the selective specificity of the compounds of this invention for retinoid X receptors, these compounds can also be used to purify samples of retinoid X receptors *in vitro*. Such

purification can be carried out by mixing samples containing
retinoid X receptors with one of more of the bicyclic derivative
compounds disclosed so that the compound (ligand) binds to the
receptor, and then separating out the bound ligand/receptor
5 combination by separation techniques which are known to those of
skill in the art. These techniques include column separation,
filtration, centrifugation, tagging and physical separation, and
antibody complexing, among others.

While the preferred embodiments have been described and
10 illustrated, various substitutions and modifications may be made
thereto without departing from the scope of the invention.
Accordingly, it is to be understood that the present invention has
been described by way of illustration and not limitation.